RESEARCH ARTICLE

Effects of agricultural production systems and their components on protein profiles of potato tubers

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A range of studies have compared the level of nutritionally relevant compounds in crops from organic and nonorganic farming systems, but there is very limited information on the effect of farming systems and their key components on the protein composition of plants. We addressed this gap by quantifying the effects of different farming systems and key components of such systems on the protein profiles of potato tubers. Tuber samples were produced in the Nafferton factorial systems study, a group of long-term, replicated factorial field experiments designed to identify and quantify the effect of fertility management methods, crop protection practices and rotational designs used in organic, low input and conventional production systems. Protein profiles were determined by 2-DE and subsequent protein identification by HPLC-ESI-MS/MS. Principal component analysis of 2-DE data showed that only fertility management practices (organic matter *vs.* mineral fertiliser based) had a significant effect on protein composition. Quantitative differences were detected in 160 of the 1100 tuber proteins separated by 2-DE. Proteins identified by MS are involved in protein synthesis and turnover, carbon and energy metabolism and defence responses, suggesting that organic fertilisation leads to an increased stress response in potato tubers.

Keywords:
2-DE / Conventional cultivation / Organic cultivation / *Solanum tuberosum* L.

1 Introduction

The agronomic practices used in intensive conventional and organic food production systems are significantly different [1]. This applies particularly to (i) fertility management (chemosynthetic mineral N and P fertilisers are the dominant N and P input in conventional systems, while organic systems rely mainly on organic matter such as legume fertility building crops or manures and compost and prohibit chemosynthetic mineral N and P inputs), (ii) crop protection protocols (chemosynthetic pesticides, fungicides, herbicides and defoliation agents are widely used in conventional systems, but prohibited in organic systems) and (iii) rotational designs (organic farming standards prescribe very

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Abbreviations: ANOVA, analysis of variance; **Hsp,** heat shock protein; **PCA,** principal component analysis

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diverse crop rotations and defined periods in which fertility building legumes are grown, while intensive conventional systems tend to use less diverse rotational or monoculture systems). 'Low input', but not organically certified production systems tend to use lower levels of chemosynthetic mineral fertiliser and/or crop protection inputs and/or use more diverse rotational systems.

Agronomic practices used in organic and low input production systems have repeatedly been claimed to deliver environmental or biodiversity, agronomic (*e.g.* reduced incidence or crop susceptibility to pests and diseases) and food quality (related to differences in food composition) benefits compared to intensive conventional systems. While there is now a significant body of evidence for environmental and biodiversity benefits associated with organic and low input systems [2], evidence for differences in crop resistance and composition of nutritionally relevant compounds is currently insufficient and often contradictory [3–6]. There appears to be no information on the effects of agricultural production systems on gene expression and protein profiles in crops, which could elucidate the underlying mechanisms for differences in crop resistance and composition. It is a general observation that organic and low input systems result in lower productivity and marketable yield. Since consumer demand and premiums achieved for foods from low input systems are to a large extent based on consumer perception that significant benefits are associated with organic foods, it is essential to identify and quantify differences between produce from organic, low input and conventional systems.

The recently developed profiling techniques allow for a comprehensive overview of the responses of the plant to external conditions. Proteins are crucial functional molecules in the cell, and most allergens are proteins. Proteome analysis is increasingly used in functional studies on plants [7]. We have recently used proteomics to address genetic differences between potato varieties [8] and differences between physiological states of potato tubers [9]. To our knowledge, proteomic profiling studies to characterise the responses of plants to different agronomic regimes have not been attempted previously.

The main objectives of this study were therefore to (i) determine the effect of crop management practices on protein profiles of potato (*Solanum tuberosum* L.) tubers and (ii) identify the proteins for which differences in the level of expression could be detected.

2 Materials and methods

2.1 Field experimental design

The Nafferton factorial farming systems study was established in 2001 on a field with a uniform sandy loam (alluvial deposit) soil at the University of Newcastle's Nafferton Experimental Farm, near Stockfield, Northumberland. The field was previously managed to conventional management practices and had followed the same 8 years rotation (2 years rye grass followed by 2 years winter wheat, barley, winter oilseed rape, winter wheat, winter barley before going back to ryegrass) for 24 years. The study consists of a series of four field experiments established within four 122×122 m^2 blocks (replicates). Each block is subdivided into four subblocks (one for each experiment) each with a size of 24×112 m^2. The position of individual experiments or subblocks within each of the four blocks was randomised. Each subblock is divided into four 6×112 m 'crop rotation' main plots, each with different rotational sequences; two rotational sequences typical for organic systems and two typical for conventional systems were established. Each crop rotation main plot was subdivided into two 6×56 m 'crop protection' subplots in which crop protection is either done according to conventional farming practices or to organic crop protection standards. Each crop protection subplot was further subdivided into two 6×24 m 'fertilisation' subsubplots in which fertilisation was either carried out according to conventional farming practice or organic farming standards.

Tuber samples used in the protein profiling experiments described here were taken in 2004 when potatoes were established in two experiments/subblocks and in four rotation main plots. One potato main plot was established either after 2 years of grass and clover (2001 and 2002) and 1 year of winter wheat (2002/2003). Three separate potato main plots were established after 3 years, 2001–2003, of grass and clover (*i.e.* all three main plots had the same rotational history).

Since all main crops had been cropped with grass and clover in 2001 and 2002 and the only difference between main plots was the different precrops used in 2003 (wheat *vs.* an additional year of grass and clover), it was possible to analyse results as a 3-factorial experiment with precrop, crop protection and fertilisation as factors, assuming a split-plot design outlined in Table 1.

Table 1. Outline of the field experimental design. The 3-factorial experiment with crop protection (either conventional or organic), fertilisation (either conventional or organic) and precrop (either grass and clover or winter wheat) as factors assumed a split-plot design, leading to eight different treatment combinations; the number of replicate samples in each combination is indicated. The crop protection and fertilisation protocols are described in detail in Supplementary Tables 1 and 2

Fertilisation	Crop protection			
	Conventional		Organic	
Conventional	Grass and clover 12	Wheat 4	Grass and clover 12	Wheat 4
Organic	Grass and clover 12	Wheat 4	Grass and clover 12	Wheat 4

2.2 Crop protection treatments and fertilisation protocols

The crop protection protocols used in crop protection subplots (Supplementary Table 1) and the fertilisation protocols used in the two fertilisation subplots (Supplementary Table 2) were the same as used by the commercial conventional (half of Nafferton farm is managed and certified according to 'Farm Assured British' conventional farming standards) and organic (half of Nafferton farm is managed and certified according to 'Soil Association' organic farming standards) farming businesses at Nafferton Experimental Farm. The conventional fertilisation protocol included mineral N, P and K fertilisers, while the organic protocol included compost. N content was roughly equivalent in both protocols.

2.3 Potato agronomy and yield assessment

Nonchitted potato seeds (variety Santé) were planted using a semiautomatic two-row potato planter (Checci & Magli, Budrio Bologna, Italy). Potato seed tubers planted in subplot 1 (conventional crop protection) were produced under conventional seed potato production conditions (Supplementary Table 1). Potato seed tubers planted in subplot 2 (organic crop protection) were produced to organic (Soil Association, Bristol, UK) seed potato production standards (Supplementary Table 2). All fertilisation treatments were applied 4 wk prior to planting of tubers and no irrigation was used. After defoliation, tubers were left in the ground for 4 wk to allow skin maturation, and then harvested using a single-row potato harvester (Ransomes, Ipswich, UK).

Potato yield was assessed by harvesting the four middle rows of each plot using a single-row harvester. The two outer rows and the first 0.5 m of each plot were not included in yield assessments. Tuber fresh weights were determined immediately after harvesting. Dry weights were determined from a subsample of harvested tubers dried in a drying oven (Genlab, Widnes, UK) at 80°C for 2 days.

2.4 Analysis of total nitrogen, phosphorus and potassium content

Tuber samples were dried at 70°C for at least 24 h and ground with an achat grinder to a fine powder. Total N was determined using the standard Kjeldahl N analysis method. For total P and K determination, 200 mg of the ground sample was then weighed in Teflon® vessels of a microwave digestion unit (CEM MARSXpress, CEM, Matthews, NC, USA). Two millilitres of H_2O_2 and 5 mL of HNO_3 were added to each sample and digested for 25 min at 1200 W in a microwave closed digestion unit. Digested samples were filtered and added to 20 mL Milli-Q water.

All samples were then analysed for P and K content with a simultaneous ICAP-OES (inductively coupled argon plasma optical emission spectrometer) equipped with a CCD detector (Varian Vista-Pro, Varian, Palo Alto, CA, USA). The instrument was calibrated with a mixed standard prepared in the same matrix of plant samples (*i.e.* 2:5:13 ratio of H_2O_2/HNO_3/H_2O v/v/v). NIST-1567a (wheat flour) and NIST-1547 (peach leaves) were used as quality control (QC) samples.

2.5 Preparation of tuber samples for protein extraction

Tubers were sampled after 8 wk of storage at ambient temperature (8–12°C). About 800 g (fresh weight) from 4–5 tubers were combined for each replicate. Two opposite eighths (to minimise gradient effects within the tuber) were removed from each tuber within a replicate and bulked together. The resulting *ca*. 200 g of fresh weight were chopped, frozen in liquid nitrogen, freeze-dried and milled, resulting in a total of 64 samples. Freeze-dried and milled potato powders were stored at −20°C.

2.6 Protein extraction and 2-DE

Proteins were extracted from *ca*. 1 g of freeze-dried powder as previously described [10]. The protein pellet was dissolved in 2-DE sample buffer containing 9.5 M urea, 2% CHAPS, 1% DTT and 0.8% Bio-Lyte 3/10 ampholyte (BioRad, Hercules, CA, USA). Protein was quantified using the BioRad Protein Assay Dye reagent. A total of 150 μg of protein was loaded on each of the 64 gels. 2-DE was performed as described by Lehesranta *et al*. [9]. Single gel analyses were made from each biological sample.

2.7 Image analysis and 2-DE data analysis

Gel image analysis was performed with PDQuest software (BioRad). Protein spot quantities were normalised to the total quantity of valid spots (*i.e.* spots manually considered to be correctly detected) to minimise possible errors due to differences in the amount of protein loaded and staining intensity. Overall, 1097 distinct spots were matched across the 2-DE gels and used in statistical analyses. Spots with quality values of 0 and normalised quantity values <1.7 were considered to be below the detection limit and not to be indicative of protein expression. Therefore, their quantity values were set to 0. All data were $\log(z + 1)$ transformed to normalise the data, and all subsequent statistical analyses were performed using these figures.

Two distinct statistical analyses were carried out. In the first analysis, principal component analysis (PCA) was used to investigate how the proteins behaved simultaneously and to see whether differences between samples which received different treatments were evident when all the proteins were considered together. PCA was performed on the spot quantity correlation matrix.

The second, more detailed analysis, began by examining each protein separately for differences between treatments using analysis of variance (ANOVA) incorporating the split-plot design. Protein spots with more than 10 (out of 64)

values below threshold were not included in the analysis described below; this left 881 protein spots for further analysis. For each main effect (cropping history, fertilisation and crop protection regimes) and interaction, a subset of proteins was selected using a false discovery rate (FDR) cut-off of 5%. This subset included protein spots with the strongest treatment patterns. A factor profile was defined as the set of estimated (from the ANOVA) mean intensities for each level of the factor. For a particular main effect or interaction, the subset was further partitioned into groups of spots with similar profiles. The data for this partitioning were derived directly from all pairwise 5% least significant differences (LSDs) among the factor levels, derived from the ANOVAs. Partitioning was based on hierarchical clustering with average linkage and Euclidean distance. This method is described in more detail in Lehesranta *et al.* [9]. Statistical analyses were performed using Genstat for Windows, 9th Edition (VSN International, Hemel Hempstead, UK).

2.8 Protein identification by HPLC-ESI-MS/MS

In-gel digestion and identification of protein spots by HPLC electrospray MS/MS (HPLC-ESI-MS/MS) was performed as previously described [9]. Tryptic peptides were separated using Ultimate/Famos capillary LC system (LC Packings, Amsterdam, The Netherlands). The sample was loaded onto a 300 μm id × 1 mm C18 PepMap (Dionex, Sunnyvale, CA, USA) precolumn with a flow rate of 30 μL/min of 0.1% formic acid and 2% ACN. After preconcentration and clean-up, the precolumn was automatically switched in-line with the PepMap C18 (3 μm, 75 μm id × 50 mm, Dionex) analytical column and the peptides were eluted with a linear gradient starting from 100% eluent A (0.1% formic acid and 5% ACN) to 30% of eluent B (0.1% formic acid and 95% ACN) in 40 min, the flow rate being 200 nL/min. The LC was connected to mass spectrometer with a nanoES ion source (MDS Sciex, South San Francisco, CA, USA) using 15 μm PicoTip (New Objective). The positive TOF mass spectra were recorded on a QSTAR XL hybrid quadrupole TOF instrument (Applied Biosystems, Foster City, CA, USA) using information-dependent acquisition (IDA). TOF MS survey scan was recorded for mass range m/z 400–2000 followed by MS/MS scans of the two most intense peaks. Typical ion spray voltage was in the range of 2.0–2.4 kV, and N_2 was used as collision gas. Other source parameters and spray position were optimised with a tryptic digest of myoglobin.

Automated spectral processing, peak list generation and database search from raw data acquired on QSTAR XL hybrid quadrupole TOF instrument was performed using Analyst QS v1.1 and ProID v1.1 software or the MASCOT Search v1.6b13 script for Analyst QS (Applied Biosystems) in combination with the MASCOT interface [11]. Identifications were done using the NCBI nonredundant protein database (Viridiplantae) as of 07/12/06 and the TIGR potato EST database (http://www.tigr.org/) as of 07/23/04. Only peptides with a charge of +2 or +3 were considered for MS/MS. Parent ion and fragment mass tolerances were 0.15 and 0.10 Da, respectively. One miscleavage was allowed. Matches of MS/MS spectra against sequences in the databases were also verified manually.

3 Results and discussion

3.1 Crop performance and nitrogen, phosphorus and potassium content in potato tubers

Rather than simply comparing products from organic or low input and conventional production systems, the aim of this work was to produce a comprehensive set of data that could also facilitate the understanding of the possibly observed differences based on the various factors of the systems. Potatoes were thus harvested from plots that had three management regimes, the primary variants being fertiliser (mineral N, P and K fertilisation as the conventional regime or compost as the organic regime), crop protection (conventional or organic) and precrops.

The yield and basic chemical content of the potato tubers were analysed to find any effects of the alternative schemes. Yields (both fresh and dry weight based) and percentage dry matter of potato crops were significantly affected by all three main factors included in the field experiment (Supplementary Table 3). Yields were higher with conventional fertilisation and crop protection practices. On the other hand, the percentage dry matter was significantly higher when fertilisation and crop protection were carried out to organic farming standards. Clover as the precrop increased both fresh weight and the percentage dry matter. There were also significant interactions between fertility management and precrop.

The total N, P and K content in potato tubers were mainly affected by fertility management. The total N content was approximately 50% and the total P content approximately 10% higher in the tubers fertilised with the conventional, mineral fertilised-based fertilisation protocol (Supplementary Table 4). However, the K content was slightly, but significantly higher in tubers from crops fertilised to organic farming standards. Crop protection also resulted in a small but significant effect on total N content, with the organic crop protection resulting in approximately 10% higher total N content.

3.2 Protein profiles of potato tubers

Proteins from potato tuber samples were separated by 2-DE. Protein profiles of all the 64 samples were very similar (Fig. 1). A total of 1097 spots were matched across the 2-DE gels and considered in the statistical analyses. PCA was used to investigate whether the agricultural treatments could be separated on the basis of tuber protein profiles. Tubers grown with either conventional (mineral fertiliser) or organic (compost) fertilisation separated along the 1st and 2nd

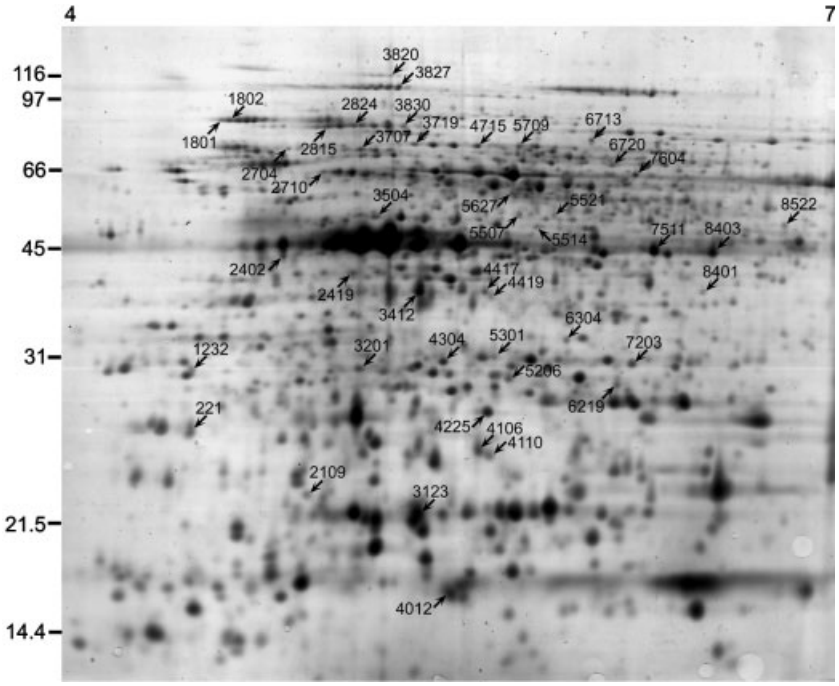

Figure 1. 2-DE image of potato (*S. tuberosum* L. cv. Santé) tuber proteins grown with the organic fertiliser regime. Proteins identified in this study are marked with arrows. The numbers refer to proteins listed in Supplementary Table 5.

principal components, which accounted for 11.5 and 8%, respectively, of all the variability in the data (Fig. 2). The fertiliser effect was independent of the other factors. ANOVA performed on individual spots highlighted significant differences between the two fertilisation regimes for at least 160 proteins selected on the basis of an FDR of 5%.

Interestingly, as far as protein profiling is concerned, tubers treated with conventional crop protection regime could not be separated from those treated with organic crop protection as assessed by PCA (Supplementary Fig. 1), nor was there any obvious effect of presowing plots with either grass and clover or wheat (Supplementary Fig. 2). Neither were there significant interactions between the three factors. Due to the dry weather conditions during the 2004 growing season, the levels of foliar blight (caused by *Phytophthora infestans*, the main disease targeted by the fungicide treatments used in both the organic and conventional crop protection regime, see Supplementary Table 1) remained below 5% in all plots. The incidence of all other diseases was also very low (data not shown). Additional work may therefore be required to really unravel any impact of crop protection methods (especially in years with higher disease pressure) and previous cropping history and rotations on the potato tuber proteome.

3.3 Proteins with significant differences between the two fertilisation regimes

The 160 protein spots with significant differences between the two fertilisation regimes showed two types of profiles: 17 were present at higher levels in tubers grown with conventional fertilisation regime, while the remaining 143 were more abundant in tubers grown with the organic fertilisation regime (Fig. 3). The number of standard error differences (SEDs) between the mean protein content in tubers grown under these two fertilisation regimes were all in the range of 2.5–5. Over 100 spots were subjected to MS for identification. Since the potato genome has not been fully sequenced, many peptides were matched against sequences from other organisms and/or ESTs (Supplementary Table 5). Most proteins were also of low abundance in the 2-DE gels, and not all of them could be identified; thus the proteins that could be identified are likely to be slightly biased towards more abundant proteins.

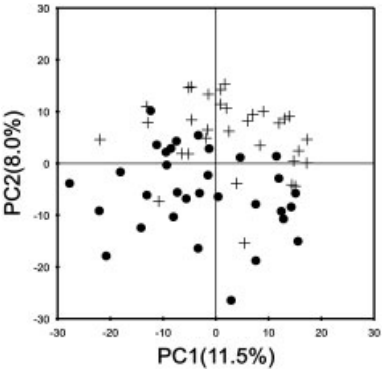

Figure 2. Principal component scores for 2-DE samples derived from potato tubers grown with the organic (+) or conventional (●) fertilisation regime. The percentage of the total variation accounted for by each principal component is shown in brackets.

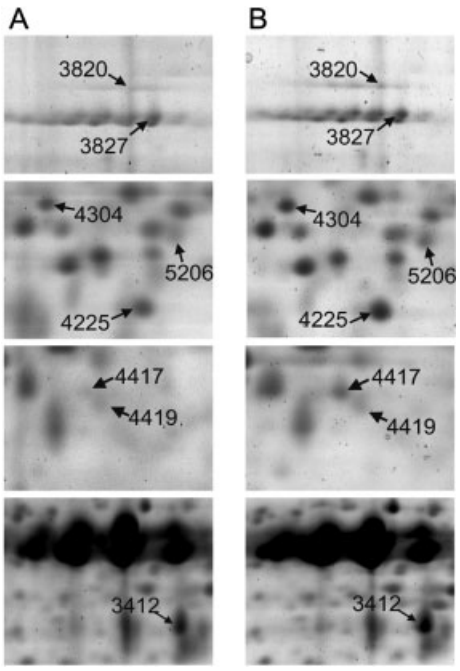

Figure 3. Examples of protein profiles with spots that showed significant differences between the two fertilisation regimes. Representative images of different sections of the gel containing tuber proteins grown with the conventional fertiliser regime (A) or the organic fertiliser regime (B) are shown. The numbers refer to proteins listed in Supplementary Table 5.

3.3.1 Proteins more abundant in tubers grown with the organic fertilisation regime

Spots that were more abundant in tubers grown with the organic (compost based) fertilisation regime included several proteins involved in protein synthesis, folding and degradation, such as various heat shock proteins (Hsps) and putative chaperonins (spots 1801, 1802, 2815, 2824, 3707, 3820 and 3827). Hsps (*e.g.* Hsp70) are known to be involved in assisting protein refolding, translocation and targeting to degradation [12]. P0 ribosomal protein-like protein (2419) and peptidylprolyl isomerase (3830) are also likely involved in protein synthesis and folding. Furthermore, several spots (1232, 4304, 5206, 5627, 6219, 7203) were matched against subunits of the 20S proteasome, a major component of protein degradation involved in development, stress responses and other cellular processes [13]. Aspartic protease (4417) has also been suggested to be involved in storage protein processing and protein degradation for nitrogen source [14]. Another hydrolytic enzyme, β-xylosidase (LEXYL2) of tomato (spot 6713), has been shown to participate in the breakdown of xylans of cell walls [15].

Several spots were matched against enzymes involved in glycolysis and energy metabolism, such as phosphoglycerate mutase (3719, 4715), glyceraldehyde 3-phosphate dehydrogenase isoforms (7511, 8403), ATP synthase subunits (2710, 7604), dihydrolipoamide dehydrogenase precursor (6720) and putative succinate dehydrogenase (spot 5709) involved in the tricarboxylic acid cycle.

Various proteins that are commonly up-regulated in stress responses were also increased in tubers grown with the organic fertilisation regime. Kunitz-type enzyme inhibitor (spot 221) is considered to assist in the protection against pest predation [16]. Superoxide dismutases (spot 4225) and ascorbate peroxidases (5301) are involved in the maintenance of redox balance and in the metabolism of reactive oxygen species produced in cellular processes such as electron transport in mitochondria or in various stress conditions [17]. Spot 3412 was matched against a hypothetical protein similar to lactoylglutathione lyases (glyoxalase I), and spot 6304 against hydroxyacylglutathione hydrolase (glyoxalase II). Both enzymes are involved in the cellular detoxification of toxic oxoaldehydes, in particular, methylglyoxal produced mainly as a byproduct of glycolysis [18]. Spot 8401 was matched against a protein similar to β-cyanoalanine synthase, which is the key enzyme in cyanide metabolism, converting cyanide and cysteine to β-cyanoalanine and sulphide in mitochondria. Its major physiological role is considered to be the detoxification of cyanide produced in tissues. β-Cyanoalanine synthase expression has been previously observed in potato tubers but the physiological importance is not clear [19]. 3-Mercaptopyruvate sulphurtransferase (spot 4419) has also been suggested to be involved in cyanide metabolism in *A. thaliana* [20].

3.3.2 Proteins more abundant in tubers grown with the conventional fertilisation regime

Proteins that were more abundant in tubers grown with the conventional (mineral fertiliser based) fertilisation regime included a small Hsp (spot 3123) and a proteinase inhibitor (4012). It is of interest that, while the small Hsp was more abundant in conventionally fertilised tubers, several large Hsps were less abundant. We have observed also previously that the small Hsps have a distinct expression pattern, in particular, when compared to the large Hsps, during the tuber life cycle [9]. Two of the spots identified as patatins, the major storage proteins of potato tuber, were of smaller molecular weight than the previously identified isoforms [8, 9] and may thus be degradation products. The higher amounts of these patatin spots may reflect slightly higher amounts of storage proteins or their increased degradation in conventionally fertilised tubers. Overall, the major isoforms of patatin with the molecular weight of *ca.* 40 000–45 000 and p*I* of 4.5–5.5 [21, 22] did not show any marked changes under the conditions studied.

3.4 General discussion

Agronomic practices used in organic and low input production systems have been claimed to deliver many benefits, while evidence for differences in the composition of the

products has been insufficient. Therefore, it is important to qualify and quantify any differences in the composition of organically and conventionally grown crops. Several reports have suggested that increased disease pressure due to the lack of use of pesticides could lead to increases, *e.g.* in the levels of polyphenol compounds, of organically produced plants of various species [23–25]. However, some have reported opposite effects, *i.e.* higher levels of secondary metabolites due to conventional crop protection [26]. Therefore, it has been suggested that if an effect exists, it may thus be small, and the effects occurring in different cultivation systems may to some extent balance each other out by imposing different types of stress [27]. Recently, Zörb *et al.* [28] reported that no major differences were found in the metabolite profiles of organically *vs.* conventionally managed wheat, although a decrease in agronomic parameters such as yield in organically grown crop due to smaller nutrient availability was observed. As to the protein profiles, the differences observed in the present experiment between the alternative regimes were also relatively small. The major source of differences in the protein profiles between conventional and organic cultivation practices was the fertilisation regime, while crop protection treatments and the previous crop in the rotation did not lead to a discernible effect.

It has been hypothesised that organic crops would generally grow under increased stress conditions because of insufficient nutrient supply [28]. The accumulation of chaperones and proteins involved in the degradation of proteins and other macromolecules suggests that protein synthesis and turnover and other hydrolytic reactions are more active in the tubers grown with the organic fertilisation regime. Furthermore, the levels of several enzymes involved in glycolysis and energy metabolism were higher in these tubers, suggesting a higher rate of cell respiration. Many of the identified proteins are also highly expressed in defence reactions, such as the Hsps [12], aspartic proteinase [14] and enzymes that function as defence mechanisms against oxidative stress (superoxide dismutase, ascorbate peroxidase). Many of these mechanisms are also known to intensify as responses to various stresses. These results therefore suggest that organic fertilisation leads to an increased stress response in potato tubers, as shown by the increased abundances of a large number of proteins previously implicated in stress responses. In agreement with this, it has been shown that compost as soil amendment can result in the activation of systemic resistance and increase in the activities of defence-related proteins [29].

Total nitrogen levels in tubers were significantly higher with the conventional fertilisation regime, although roughly similar amounts of nitrogen were applied with both the organic and conventional fertilisation protocol. However, while the mineral nitrogen fertiliser applied with the conventional fertilisation regime was water-soluble and thus immediately available for the plant, the nitrogen supplied with the compost contained less than 1% water-soluble NH_4 and NO_3 and was mainly in the form of organic nitrogen (data not shown). Nitrogen supply pattern to potato crops during the growing season was therefore expected to be very different between the two fertilisation regimes. With the mineral fertilisation, all nitrogen applied will be immediately available for the plant after application and available nitrogen levels will have subsequently declined due to uptake by plants and to nitrogen losses, *e.g.* by NO_3 leaching and denitrification [30]. The organic matter-based nitrogen supplied in the form of compost will only become available subsequent to mineralisation of the organic matter by the soil microflora, which is known to increase with soil temperature [31]. Nitrogen supply for compost is thus expected to have been very low immediately after planting and have subsequently increased throughout the growing season. Due to this and the fact that only part (for manure usually around 50%) of the nitrogen from organic matter becomes available for plants in the first growing season [30], it is likely that the nitrogen availability from similar levels of compost inputs will gradually increase when organic fertilisation protocols are used, which is also likely to affect the expression of proteins that are closely linked to nitrogen availability pattern.

Nitrogen metabolism is complex and frequently linked to carbon metabolism and glycolysis [32, 33]. Nitrate fertilisation is frequently observed to lead to higher levels of protein and also to changes in carbon metabolism, including an increase in organic acids [34]. Microarray analyses have shown that rice plants stressed with low N responded with both up- and down-regulation of many disease and defence-related transcripts [35]. Furthermore, transcript levels for many genes involved in redox metabolism and processes such as glycolysis and cell wall modification respond to nitrogen status in *Arabidopsis* [36]. Although the effects of the two fertilisation regimes explored in the present study are likely to be complex, the differences seen could therefore, at least in part, be due to differences in nitrogen availability. The nutritional status of the plant can also have an effect on the capability of the plant to respond to stresses [29].

Several potato proteins, including the major storage protein patatin [37] and Kunitz-type protease inhibitors [38] have been shown to have allergenic properties. In this study, only one patatin isoform was slightly less abundant in tubers grown with the organic fertilisation regime, while no significant changes in the relative abundance of the major patatin isoforms were found. On the other hand, one spot matched against a Kunitz-type protease inhibitor was more abundant. Therefore, it appears that the different cultivation techniques did not lead to any major differences in the levels of allergenic proteins identified in this study.

4 Concluding remarks

Protein profiling of potato tubers by 2-DE was used to analyse differences caused by organic *vs.* conventional cultivation practices. These results show that the major source of difference in the protein profiles between conventional and

organic cultivation practices is the fertilisation regime, while crop protection treatments and the nature of the previous crop in the rotation do not appear to lead to a discernible effect. Furthermore, the identified proteins suggest that organic fertilisation leads to an increased stress response in the tubers. While the effects of the cultivation techniques on the protein pattern are much smaller than, *e.g.* differences between different cultivars [8], this approach illustrates the capacity of proteomic techniques to detect subtle differences even in large field experiments.

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