Proteomic Analysis of Plant Response to Ionising Radiation in the Context of Plant Stress Response

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INTRODUCTION

In the light of global environmental changes it becomes increasingly important to understand how organisms can react to changing conditions, including those that are potentially stressful. A better understanding of how plants respond to and manage abiotic stress could help predict suitability to changing habitats and help understand how a plant may respond to a new stressor. All living organisms share 44 cellular stress response proteins which founded the stress response proteome 3 billion years ago (Kültz 2005), it is evident that responding to and managing abiotic stress was a vital step in cellular evolution. With these stress proteins in common, knowledge gained in the cellular stress response of one species could aid in research of another species.

Responses to ionising radiation are often observed after the treatment of very high doses (Costa-Nunes et al, 2006; Wada et al, 1998). This shows a cellular stress response to ionising radiation as a stressor, but not necessarily the cellular stress response to all doses or all ionising radiation stressors. It has been shown that organisms respond differently to different exposure rates of the same abiotic stressor (Kovalchuk et al, 2007).

As such, data from high ionising radiation dose stress response experiments may not represent the stress response to ionising radiation doses received in the environment, such as that received by flora surrounding nuclear power stations.

It has been suggested that plant responses to ionising radiation would be minimal at low doses because life evolved during a period when the average Earth background dose was higher than it is today. Although Earth has decreased in radioactivity over the last 4 billion years due to nuclear decay, the background dose from beta gamma was not above 7.1 mGy y^{-1} (Karam and Leslie, 1999) 4 billion years ago which is equal to only 1738 Bq. This is less than some places on Earth today.

When investigating the stress response of an organism to any stressor it is important to study the proteomic response in addition to the genomic response. It is often more difficult to observe protein changes compared to mRNA extraction and gene expression analysis, especially without the ability to amplify levels of low abundance proteins, as with PCR. Genomic analysis alone however, does not fully reflect the cellular state because it has been shown that mRNA level changes do not always correlate with protein level changes (Anderson and Anderson 1998; Alemán et al, 2007).

MATERIALS AND METHODS

Plant Material and Treatment

Arabidopsis thaliana Columbia O ecotype seeds obtained from the Nottingham Arabidopsis Stock Centre (Scholl et al., 2000) were surface sterilised in 70% ethanol for 3 minutes followed by a 3 minute rinse in sterile distilled water. Seeds were sown in a pipette tip of 450µl nutrient agar as per Norén et al. (2004) and after a 4 day cold treatment to synchronize germination, were allowed to germinate in a growth cabinet (Sanyo MLR – 350HT Nijverheidsweg, Netherlands) Settings were 13 hour day at 22ºC, 11 hour night at 18ºC with an average humidity of 65% and average light intensity of 109 µmol photons $m^{-1}s^{-1}$.

Fourteen day old seedlings were transferred to a polystyrene float in 1 litre hydroponic solution (135 x 203 x 60 mm container) which was changed fortnightly. At 34 days old, plants were treated with 90, 000 Bq/l ^{137}Cs by direct addition to the hydroponic solution. Shoot and root were harvested separately at 20 minutes, 28 hours, 48 hours and 72 hours after treatment. (0.01 mGy, 1.09 mGy, 2.01 mGy, 3.02 mGy respectively)

Sample Preparation

Protein extraction as Giavalisco et al. (2003). Protein from plant root and shoot were extracted separately. Extracted protein was precipitated by the addition of equal amounts of 20% trichloroacetic acid in acetone before centrifugation at -20ºC for 1 hour. Pellet was resuspended in an 8M urea, 4% CHAPS solution.

Protein Separation by 2D Electrophoresis

Two dimensional electrophoresis was carried out using the Ettan Dalt system and the method described in the Amersham 2DE Handbook (Berkelman and Strenstedt, 1998). Gels were scanned for image analysis and the number of visible protein spots were recorded.

RESULTS AND CONCLUSIONS

The number of protein spots observed by two dimensional electrophoresis are presented for *A.thaliana* shoot and root samples. There was no significant change observed between the number of spots from the control and exposed shoot samples for any of the exposure times (Fig.1). Exposure to 3 mGy and less via the roots does not produce easily detectable changes in the shoot proteome of *A.thaliana*. However, a change was observed in the root proteome (Fig. 2). Root samples from 20 minute ^{137}Cs exposed plants showed an increase in visible protein spots which decreased for subsequent exposure times. The number of visible protein spots for the root sample of plants exposed for 72 hours was no different to that of the control root sample. Exposure to 2 mGy and less via the roots does produce easily detectable changes in the root proteome of *A.thaliana*.

The method of protein extraction and electrophoresis was thus adequate to demonstrate changes in protein expression in *A.thaliana* is exposed to ^{137}Cs. The largest difference between protein spot numbers was found in root samples from plants exposed for 20 minutes. Experiments are currently underway to investigate a) proteome changes of *A.thaliana* root after exposure to 90,000 Bq/l ^{137}Cs for a range of exposure times below 28 hours, b) the dose

response of ^{137}Cs exposed roots and shoots of *A.thaliana*, c) the response of plants exposed over the generations. MALDI-ToF and Q-ToF will be used to identify proteins with changed expression in all these experiments.

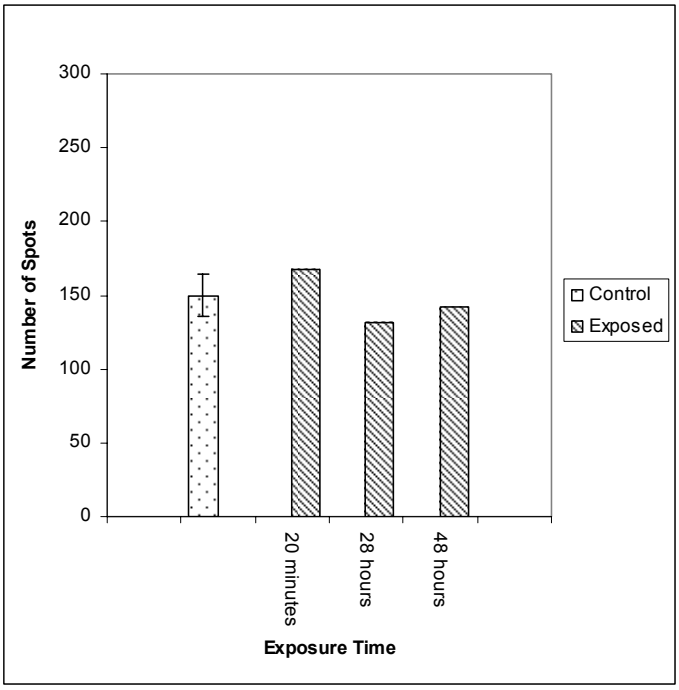

Figure 1. Number of protein spots observed by two dimensional electrophoresis in the shoot of control and ^{137}Cs exposed plants.

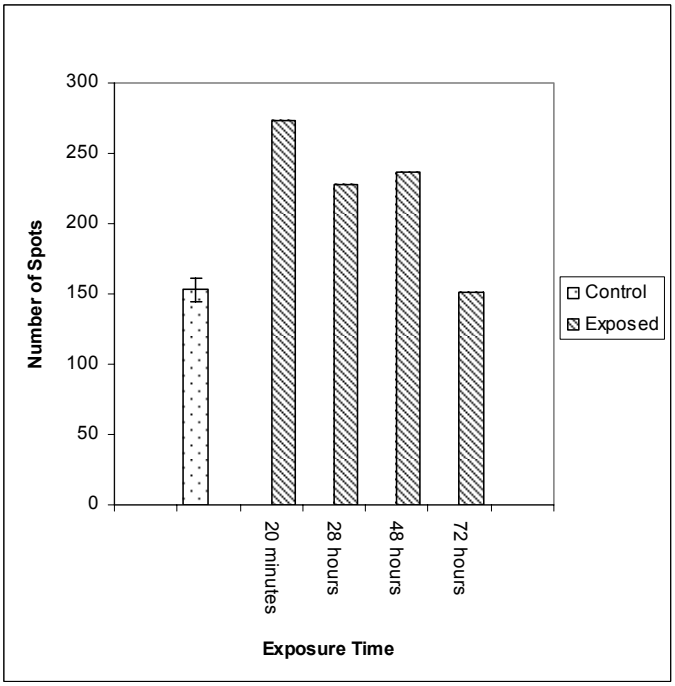

Figure 2. Number of protein spots observed by two dimensional electrophoresis in the root of control and ^{137}Cs exposed plants.

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