Environmental Change

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Environmental change is one of the most serious threats facing our society and its effects will have major impacts on many sectors of the economy. The Scottish Government has set the challenging target of reducing greenhouse gas (GHG) emissions by 80% by 2050 and it is estimated that the land-use sector contributes about 20% of the total GHG emissions. At SCRI, research to improve fertiliser management, conserve organic matter in soils and the soil's ability to store carbon is aimed towards reducing GHG emissions. Work to increase knowledge of the effect of changing climate on the geographic range and abundance of pests and pathogens will better prepare us to counter new or emerging threats to crops.

Similarly, increased knowledge of the effect of abiotic stresses such as heat, cold and drought on the plant's ability to mount disease defence responses, and their impact on crop quality, will help us to develop the tools and technologies to mitigate, adapt and manage the effects of such changes in Scottish agricultural systems. Here we present four articles that provide a snapshot of some of our work. The articles illustrate the wide ranging nature of the work and include using plants to stabilise slopes thereby preventing landslips or soil erosion and investigating what makes grass plants drought tolerant. We also report on research to understand the environmental influences on pathogenicity and the effect of abiotic stress on mRNA processing by alternative splicing leading to variation in the proteins produced.

Green engineering for sustainable environmental solutions

A. Glyn Bengough[1], Paul D. Hallett, Ken Loades, Blair M. McKenzie & Ron Wheatley

Increasing recognition of the ecosystem services provided by the environment to both urban and rural populations is particularly relevant to the interface between the plant-environmental sciences, and civil engineering. The needs of society for access to fresh water and food and to a healthy, biologically diverse, and aesthetically pleasing environment are highlighted in recent policy documents that also require greatly reduced disposal to landfill and transition to a low-carbon economy. These policy drivers are in addition to, and a consequence of, natural drivers associated with global environmental change.

The interface between plants and engineering is the subject of considerable interaction between SCRI and the University of Dundee, including a joint senior appointment with the Division of Civil Engineering in 2010 to further develop this area, and complement existing links between SCRI, the College of Life Sciences, and the Centre for Environmental Change and Human Resilience (CECHR). The majority of research has concentrated on quantifying biophysical interactions between roots and the soil, including the stabilising effects of plant root systems on soil slopes, to decrease the likelihood of shallow landslips. (Fig. 1) The increase in soil strength due to the direct mechanical reinforcement of soil by roots has been measured for both woody and thin fibrous root systems, and mathematical models have been tested that relate root reinforcement to root number and diameter distributions. Predicted increases in the frequency of intense rainfall events may render more slopes vulnerable to erosion and landslips, and a climate chamber has been constructed at the University of Dundee to enable this area of research on scaled models of soil slopes. (Fig. 2) The quality of output of this collaboration has been recognised (e.g. 'Outstanding Paper Award', European Society of Agricultural Engineers 2008; nomination for Institution of Civil Engineer's Award in Engineering Sustainability, 2010), has led to invited reviews, and created links with research institutions and companies throughout Europe.

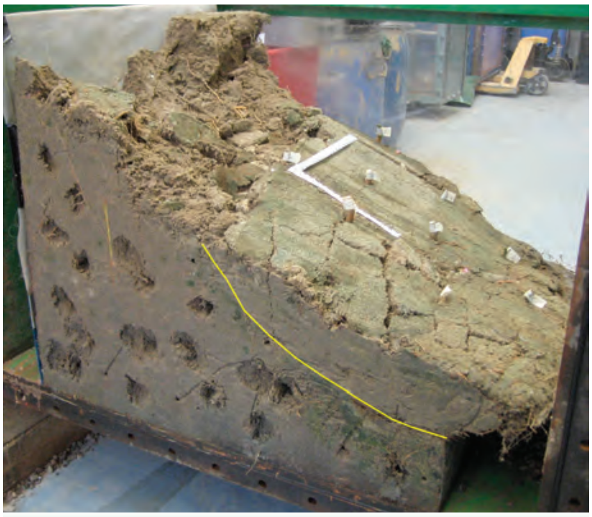

Figure 1 Model of rooted slope following testing in the centrifuge at 15 x gravity. Yellow line indicates failure plane.

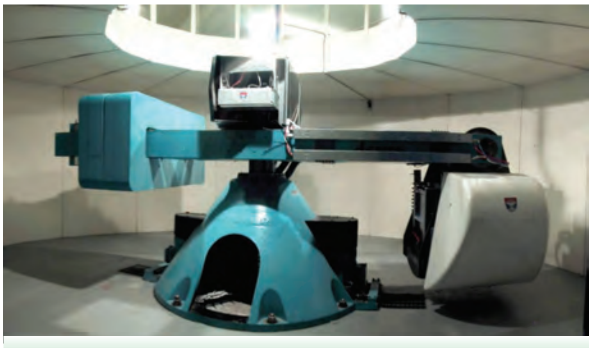

Figure 2 University of Dundee geotechnical centrifuge used to test scaled models of slopes at up to 100 x gravity.

Research encompasses both applied practical solutions, and more fundamental research. For example, the role of bulky organic waste in aiding plant establishment on embankments has been highlighted in a recent study with WRAP, that aimed to use processed waste materials for environmental benefit along our transport corridors. This work, in association with Dundee City Council, showed that grass establishment was enhanced, and surface runoff and soil erosion decreased on plots that were amended with quality green compost. Surface application of the compost was the most beneficial. (Fig. 3)

At a much smaller scale, the mechanics of soil deformations around roots is being studied both

[1] Joint with Division of Civil Engineering, University of Dundee

Figure 3 WRAP slope at Riverside, showing amendment with quality green compost enhancing grass establishment.

theoretically and experimentally. This work is showing us how plant roots deform the soil as they grow, how root hairs may help to anchor the growing root tip, and how organisms such as earthworms penetrate the soil. Our future aim is to build on this work at both fundamental and applied ends of the spectrum, to deliver both increased understanding of basic mechanisms, and practical solutions to real problems.

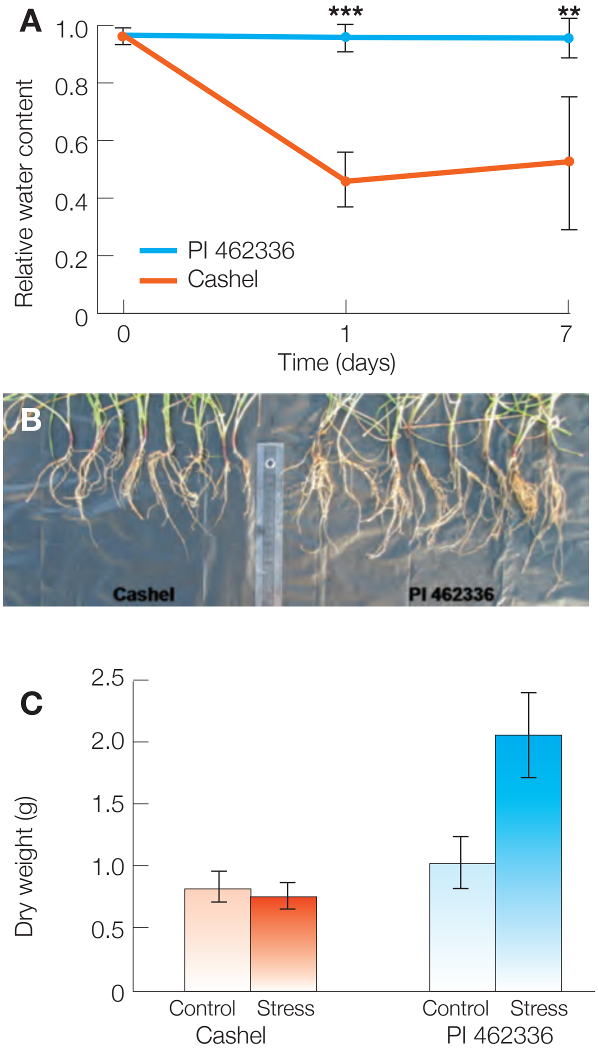

Figure 4 Physiological response of perennial ryegrass to water deficit. (a) Relative water content (RWC) levels of the leaf tissue after 1 week of stress (** $P<0.01$. ***$P<0.001$ $n=6$). (b) Root biomass of PI 462336 in comparison to Cashel under polyethylene glycol (PEG) induced drought stress after 2 weeks of water-limitation. (c) Root dry weight of Cashel and PI 462336 genotypes under control conditions and PEG induced drought stress after 2 weeks.

The impact of climate change on grasses: ryegrass and drought tolerance

Alexandre Foito, Stephen L. Byrne[1], Tom Shepherd, Susanne Barth & Derek Stewart

Climate change predictions for the north and west regions of Europe suggest a slow increase of basal temperature with an increase in extreme weather events, such as floods and droughts, consequently increasing stress experienced by crop plants. Considering that one of the major environmental factors which limits the growth and productivity of plants in the world is water-limitation, it is important to improve the ability of plants to cope with such stresses in order to at least maintain, if not increase, their current productivity.

The majority of agricultural land in the UK is devoted to grasslands, with *Lolium perenne* (perennial ryegrass) being the major grassland species present. This species possesses high yields and digestibility compared to other grass species, hence it is an economically important forage for animal nutrition. However, perennial ryegrass is susceptible to abiotic stress conditions.

Water deficit in particular was the focus of a collaborative project with The Irish Agriculture and Food Development Authority (TEAGASC), which aimed to investigate the molecular and biochemical mechanisms which are responsible for increased tolerance to water limitation. Two genotypes, with contrasting drought response, were

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exposed to one week of water limitation which resulted in the decrease of the water content in the leaf tissues of the non-tolerant genotype (Cashel) whereas in the tolerant (PI 462336) variety water content appeared to be unaffected by the water deficit treatment (Fig. 4).

A comprehensive metabolomic analysis was carried out for root and leaf tissues and major differences were observed. These included an overall reduction of metabolite levels (particularly fatty acids) in both tissues of Cashel upon exposure to stress, which suggested that water limitation was constraining source activity in the plant – possibly by affecting the photosynthetic machinery. In contrast, PI 462336 accumulated sugars and compatible solutes such as trehalose, glucose, fructose, maltose and raffinose in response to water deficit. Raffinose in particular exhibited the highest increase in both leaf and root tissues where it is thought to act as an osmolyte and potentially aid in scavenging radical oxygen species. Therefore, it appears that raffinose levels play an important role in improving tolerance to water limitation in *L. perenne* and may eventually become a target for the selection of superior drought tolerance in other crop species.

In addition to the metabolomic approach a complementary transcriptomic approach was used for the tolerant variety and revealed a number of relevant genes regulated by water deficit. These included a dehydration responsive element binding (DREB) transcription factor, an aquaporin (involved in water transport across membranes) and a gene encoding a fructan:fructan 6-G fructosyl transferase (6G-FFT), all of which have well documented roles in drought tolerance.

The results of expression of 6G-FFT prompted further experimentation in order to determine whether fructans (polymers of fructose) may be responsible for the difference in tolerance between varieties. Comparison between genotypes revealed that the tolerant genotype had 1000-fold higher expression levels in the leaves, suggesting an important role of fructans in drought tolerance. Fructan contents and polydispersity were characterised and subtle differences were observed between genotypes which were attributed to the presence of higher molecular weight fructans in the tolerant genotype. However, the contribution of this biochemical change towards improved water limitation tolerance is not well understood.

Overall, this work highlights some of the mechanisms which may affect tolerance to water deficit periods and hence may provide target traits for the selection of enhanced tolerance to drought.

Asymptomatic infection – the Trojan horse of crop production

Adrian C. Newton, Anna Avrova, Amar Thirugnanasambandam & Mark Looseley

As plant pathologists we tend to focus on diseases and their causal agents. In barley the two we concentrate on at SCRI are ‘rhynchosporium’ or ‘leaf scald’ caused by *Rhynchosporium secalis* and ‘ramularia leaf spot’ caused by *Ramularia collo-cygni*. Molecular detection methods such as quantitative PCR have shown us that both of these fungi are present on many barley crops most of the time, but as macroscopically-symptomless infections. Transformation of these two pathogens with green fluorescent protein allowed live infections to be viewed microscopically. It has shown how extensive such infections are, enabling spores to be formed and the whole lifecycle completed without triggering disease symptoms (Fig. 5).

The importance of such observations is that they probably reflect the norm: the environment is packed with numerous viral, bacterial and fungal microorganisms not causing disease symptoms. However, given certain triggers when on suitable hosts, many of these organisms can lead to symptom development. We do not yet understand most of these triggers, but many are likely to be environmental stress factors. Whichever way climate change affects our environment, it will result in different abiotic stress factors or changed timing of stresses such as heat, cold snap, flood or drought. While our focus has often been on new invasive pests and pathogens, equally important will be maintaining and improving the ability of plants to defend themselves against existing pathogens and potential pathogens in the environment.

A corollary to the observation of extensive non-symptomatic infection is that plant breeding is normally against expression of disease symptoms and not against pathogen infection. That infection and symptom expression are highly correlated is both to be expected and fortuitous, as such selection is relatively straightforward and effective. However, symptomless infection may itself a) be reducing yield, as it must take some resources and could be inducing some defence reactions, b) mean that infection can become symptomatic if triggered, thereby increasing risk, and c) maintain a pool of pathogen variability and transmit inoculum to new environments. Furthermore, resistance against infection rather than symptom expression may use different mechanisms and be under separate genetic control and therefore offer unique (and potentially more effective) lines of defence. There must be some correlation in all cases as the pathogen must be present to form symptoms. Nevertheless, we investigated whether resistance to symptoms and infection by *R. secalis* mapped to separate genetic loci. We found that while some loci controlled both types of resistance, one locus was more effective against symptomless infection. This has given us a new target for breeding that we are actively pursuing with commercial breeders.

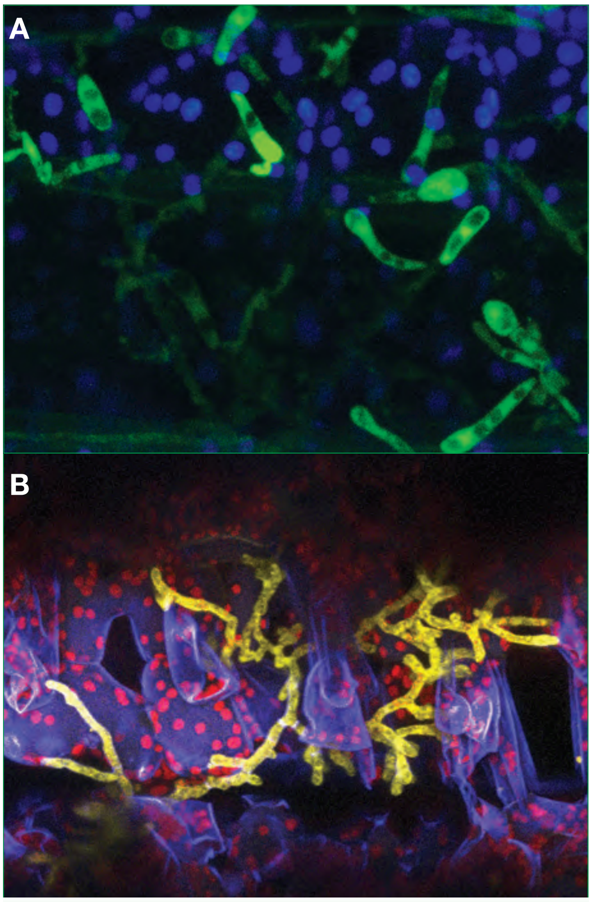

Figure 5 Confocal images of green-fluorescence protein-transformed *R. secalis* and *Ramularia collo-cygni*.
A: Sporulation of *R. secalis* during asymptomatic infection.
B: *R. collo-cygni* growing asymptomatically inside a barley leaf stained with calcofluor.

The ‘other side of the coin’ is what changes in the pathogen when it progresses from non-symptomatic growth to full pathogenic development, producing visual symptoms? Recent sequencing of the *R. secalis* genome and genes transcribed by the pathogen at the start of infection allowed identification of potential pathogenicity factors, including effectors, affecting disease symptoms development. It was followed by expression profiling of potential pathogenicity factors throughout the infection. Further functional genomics will allow identification of genes essential for pathogenicity. They will also help with understanding pathogens’ interaction with different host resistance genes. This information can lead to targeting resistance against essential processes and gene products in the pathogen that it is unable to avoid producing.

The presence of asymptomatic infection and potential genetic differences between cultivars also has implications for crop protection strategies. With some cultivars it may be more cost-effective to spray fungicide earlier than normal to reduce risk of symptom development later by knocking-out asymptomatic infections. In other cultivars the asymptomatic growth may have minimal risk of becoming symptomatic and therefore enables the decision not to spray as the risk of disease is low.

Understanding the mechanisms triggering symptom expression, whether host- or pathogen-mediated, along with the yield cost of symptomless infection, is important for devising multi-component, diverse and durable resistance breeding and deployment strategies. These are essential for responding to environmental change as we cannot necessarily identify what pathogens will threaten our crops in future.

Alternative splicing in response to abiotic stress

Craig G. Simpson, John Fuller, Dominika Lewandowska, Diane Davidson & John W.S. Brown

Gene expression involves extensive post-transcriptional processing of RNA messages before they are translated to functional proteins and ultimately influence phenotypic traits and responses. Plant messenger RNAs (mRNAs) are interrupted by non-coding sequences called introns that must be accurately and efficiently removed from the mRNA. The remaining protein coding regions, called exons, are spliced together to form the message that is translated to the functional protein. Alternative splicing (AS) occurs through alternative selection of splice sites on mRNAs derived from the same gene leading to the formation of different functional proteins (Fig. 6). The abundance of the protein variants produced by AS can differ in different plant tissues, stages of development and conditions, modulating and fine-tuning expression in developmental, signalling and metabolic pathways. In particular, AS in plants has been shown to respond to biotic and abiotic stress. In plants, the explosion in global genomic and transcriptomic sequence analysis is leading to a vast expansion of available gene sequence information and is showing that AS in plants occurs extensively. Current estimates suggest that AS occurs in at least 60% of plant genes. AS in plants is, therefore, far more important than previously thought and will have a major impact on plant gene expression.

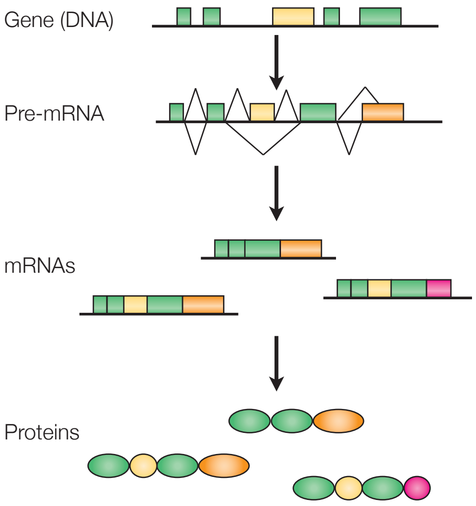

Figure 6 Schematic drawing showing the formation of alternatively spliced mRNAs and alternative proteins. Plant genes are interrupted by intron sequences that separate protein coding exons. Precursor transcripts are alternatively spliced (diagonal lines) to join different combinations of exons or parts of exons (rectangles) to give different mRNAs which are translated into different proteins.

How plants respond to and tolerate external stresses is a key question in plant physiology which impacts crop productivity. Stress responses are regulated at both the transcriptional and post-transcriptional levels. Genetic screens to identify genes involved in abiotic stress acclimation or tolerance have recovered a number of proteins known to interact with RNA. This link between environmental cues, such as abiotic stresses, and RNA metabolism is likely to reflect processes underpinning RNA production, export and stability, including AS.

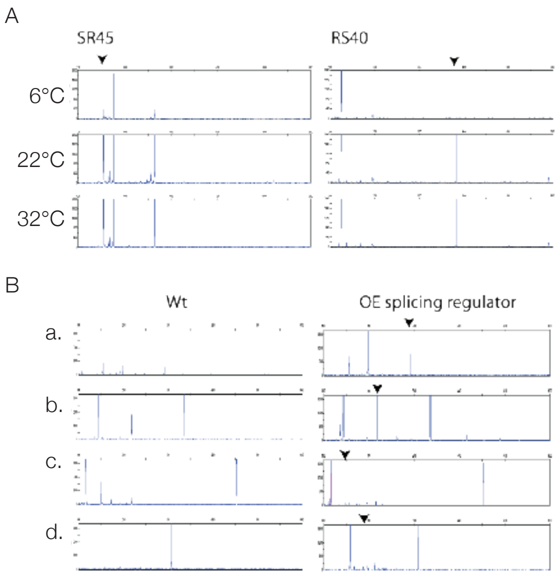

Figure 7 A high-resolution AS panel monitors changes in plant alternative splicing. Each peak in the boxes represents a different alternatively spliced transcript. Arrow heads indicate alternatively spliced transcripts that change in response to the different treatments. A. Plants grown at 22°C were given a treatment of cold (6°C) or hot (32°C) for 6 hours before isolating RNA and performing RT-PCR. Two examples of known alternative splicing regulators (SR45 and RS40) from the panel of alternative splicing events show changes in alternative splicing patterns in response to the change in temperature. B. Over-expression of the splicing regulator RS31 leads to changes in alternative splicing in stress related genes. a. Squamosa promoter binding protein-like 16 (SPL16); COLD. b. DNAJ N-terminal domain-containing protein; HEAT SHOCK. c. drought-induced-19-like; DROUGHT. d. SNF1-like protein kinase (AKin11); HYPOXIA.

AS is over-represented among genes which are up- and down-regulated under stress conditions and is thought to be one of the key points at which rapid changes in expression of proteins in response to external stimuli occurs.

The RNA processing group at SCRI has developed a high resolution reverse transcription-polymerase chain reaction (RT-PCR) system that allows us to measure dynamic changes in AS in around 300 plant genes (>900 AS transcripts). These genes represent important regulatory genes and genes with roles in responses to abiotic stresses. We are studying how AS events in these genes are regulated in response to environmental changes and to determine whether such molecular mechanisms are conserved in different crop plant species. To do this, we measure the levels of alternatively spliced variants in response to variable abiotic stresses such as, for example, temperature. We have identified genes which are transcriptionally regulated by temperature, but also have considerably altered AS patterns. Significantly, genes that regulate AS of other genes were shown to respond directly to temperature, particularly in the cold (Fig. 7A). The effect of such factors on the AS of downstream genes can also be investigated using plants that either over-express or reduce expression of these proteins. Altered expression of these regulators has a significant effect on the AS of a number of different genes including those that are important stress response genes (Fig. 7B). This system allows us to identify whether plant splicing factors promote or inhibit selection of alternative splice sites and identify their gene targets.

It is now clear that AS has a key role in regulating processes such as abiotic stress responses and will impact crop development and productivity. There is, therefore, a need to capture AS information in crop systems and as plant scientists, we have major challenges ahead to appreciate fully the importance of AS in regulating plant growth and development, its response to environmental conditions and to incorporate AS into our thinking.

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