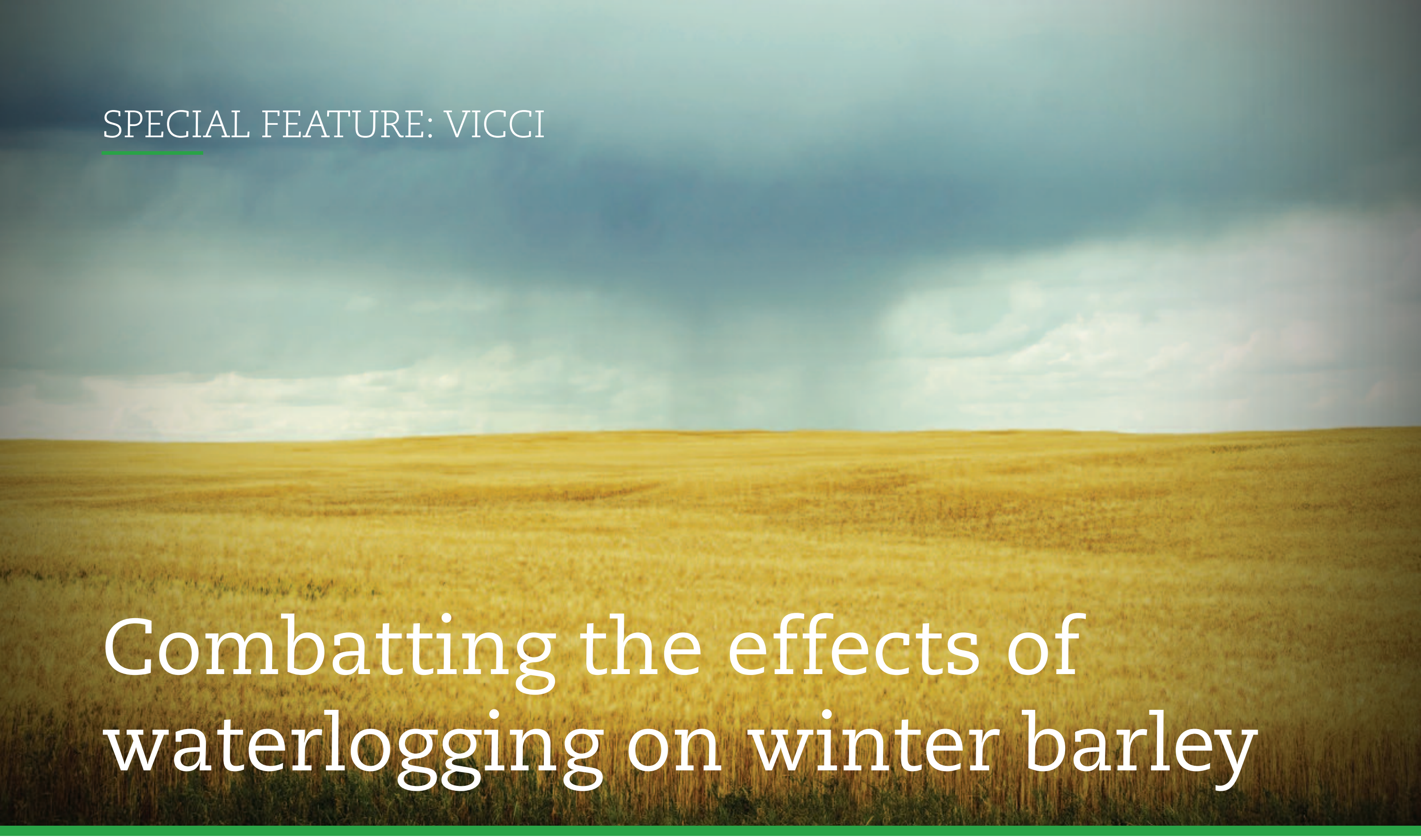

Combatting the effects of waterlogging on winter barley

As flooding events increase, TEAGASC researchers are looking at genotypic differences among winter barley cultivars with the aim of reducing the impact of waterlogging.

Flooding is becoming an increasingly challenging problem in Ireland due to global climate change-associated increases in annual rainfall. Flooding is a complex abiotic stress, which creates a low oxygen (hypoxic) environment for the plant. With increasing severity of flooding, the environment can become anoxic (no oxygen). Hypoxia and anoxia reduce available energy for the plant, decrease the rate of photosynthesis, limit nutrient uptake, and increase toxic metabolite production from the soil microbiome. These conditions are detrimental to the plant and result in crop death, as well as yield and total biomass loss. Recent climate reports predict rainfall increases of 15-20% in Ireland by 2100, highlighting the need for a solution to flooding stress. Because many crops are highly sensitive to flooding, the development of new cultivars with increased tolerance to waterlogging is essential to ensure stable crop yields.

We set out to investigate the genotypic response of winter barley towards waterlogging stress as experienced under typical early spring conditions in both field (**Figure 1**) and controlled climate chamber experiments. A barley association mapping family panel from the James Hutton Institute, which has been used in several association mapping projects in the UK, was used for our experiments. Through these activities, a set of approximately 400 winter barley cultivars has been assembled to represent the elite breeding pools of north western Europe, combined with some key progenitors and cultivars from Mediterranean Europe. The set contains nearly all the lines that completed at least two years of UK National List trials in the period 1993-2006. Inclusion of other cultivar sets extended the collection to older cultivars and other key production regions of Europe.

We observed, in flooding field trials, significant reductions in total biomass, grain biomass and height in winter barley. A 45% reduction in biomass was observed under waterlogged conditions, with total crop death resulting from the most severe treatments applied. This highlights the severe consequences of flooding stress for Irish barley production.

Mapping flooding tolerance

Phenotypic characterisation of chlorosis, necrosis and wilting allowed us to identify tolerant and sensitive cultivars. We developed a phenotypic scoring system that enabled us to select a subset of cultivars for further characterisation, under controlled conditions, of a range of shoot and root traits that may contribute to flooding tolerance, as well as molecular events. Significant reductions in chlorophyll pigment correlating with chlorosis of the leaves due to flooding were observed. This was coupled with an increase in carotenoid pigment. To further understand the underlying molecular responses to flooding in barley, we carried out RNA sequencing experiments and observed significant transcriptomic reprogramming. To further understand the extent of this reprogramming, we carried out bioinformatics analyses to functionally group these genes. As expected, our results highlighted the involvement of genes implicated in flooding responses, including alcohol dehydrogenases, pyruvate decarboxylase, sucrose synthases, and ethylene response factors, which highlights the validity of our datasets.

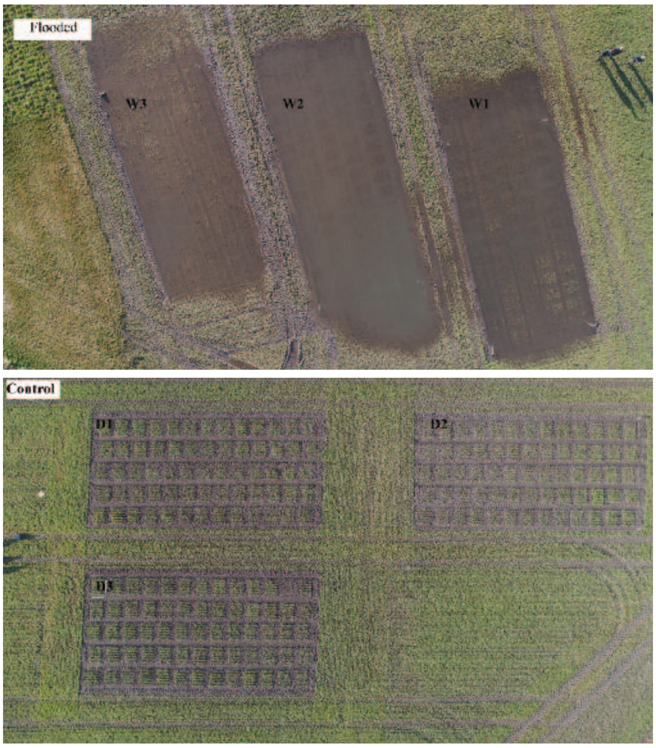

FIGURE 1: Flooding in field – year 2. Images of the blocks were taken using a DJI phantom 4 on January 26, 2018.

Interestingly, through a detailed analysis of roots, we found significant aerenchyma formation in flooded plants, with better-developed aerenchyma in tolerant barley cultivars. Aerenchyma are interconnected gas-conducting intercellular spaces that provide plant roots with oxygen under hypoxic conditions. Importantly, we have demonstrated the use of X-ray computed tomography (CT) scanning for identifying aerenchyma in intact roots. Using this technology for aerenchyma identification represents an innovative technical advance and highlights its potential application for future research and plant breeding.

New methods will assist in identifying flooding stress traits

Modern agriculture faces many issues, but there are none more pressing than global climate change. As we approach increasing climate variability, we must adapt our farming systems, and crop species and cultivars, to cope with atypical climate conditions, including tolerance to flooding. Modern plant breeding allows us to improve our crops at accelerated rates. Unfortunately, as flooding tolerance is a complex trait and reproducible screening approaches for flooding tolerance are technically challenging to set up, testing for flooding tolerance has as not yet entered as a criterion into breeding programmes and national list trial testing. With our work, we have shown that there is huge genotypic scope for barley improvement towards flooding stress as a trait, and we have also developed approaches and methods to evaluate this trait reliably, including visual markers (**Figure 2**), tissue phenotyping approaches (X-ray CT), molecular transcription-based approaches and marker genes.

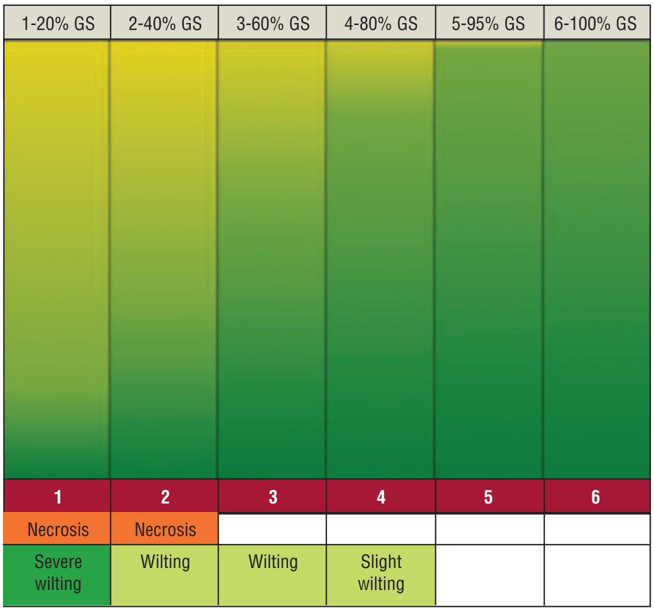

FIGURE 2: Scoring chart used for phenotyping. A colour gradient represents the percentage leaf green space (GS) indicating chlorosis in sensitive cultivars (score 1) to tolerant cultivars (score 6). The presence of necrosis on the leaves signifies sensitive cultivars. Wilting occurs on scores 1 to 4. Score 6 represents a tolerant cultivar with no phenotypic response to flooding. Score 6 is also the common phenotype of control plants.

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