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Understanding asparagus tip breakdown postharvest at a molecular and cellular level

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Abstract

Asaparagus has a very limited season in the UK and a short shelf-life. Tip breakdown occurs sporadically during chilled storage and limits shelf-life. It is characterised by internal browning and is accompanied by an unpleasant aroma. The change in aroma can be the first sign of the breakdown, and results in waste as whole batches of asparagus need to be discarded. Data suggest that the breakdown may not be initiated by microbial action, but the underlying mechanisms are yet to be fully elucidated. Tip breakdown occurs more frequently in faster growing spears when growing temperatures are higher. To explore whether the faster growth is associated with larger cells, cell size was compared in spear tips of a variety which is more susceptible to tip rot, 'Aspalim', and a variety in which tip rot very rarely occurs, 'Early California'. However, no significant differences were found. Transcriptomes were sequenced from healthy spears, and spears in which symptoms of tip breakdown could be seen visually, to assess changes in gene expression during the development of the tip breakdown. More genes were upregulated in spears showing tip breakdown compared to healthy spears after 14 days of chilled storage. The largest functional group of differentially expressed genes is related to DNA binding and transcription regulation which may provide useful information on the overall regulation of the breakdown process.

Keywords: cell size, gene expression, transcriptome.

INTRODUCTION

Asparagus is a high value perennial crop, which has increased in popularity over recent years. The UK has a yearly production of over 5,000 tonnes (Asparagus Growers' Association-AGA, 2020). However, the UK asparagus season is short: from mid-April to mid-June, resulting in imported asparagus for the remainder of the year. In 2019, over 13, 000 tonnes of asparagus were imported to the UK, mostly by air freight (Defra, 2020) with implications for CO_2 emissions. Currently, asparagus spears grown in the UK are stored chilled at 2 ˚C for only about 1 week prior to providing a 7-day shelf-life in retail outlets, where they generally experience temperatures of around 7-10- ˚C. Following harvest, spears are typically tied into bundles, and minimally processed by trimming, washing, and packaging in trays covered with plastic film. Thus, the tissues remain metabolically active (Papadopoulou et al., 2001). Perhaps because of their high metabolic activity, asparagus suffers from such a short shelf-life resulting in substantial waste at times of peak production, when it is difficult to manage asparagus yield and consumer demand.

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The most important asparagus quality attributes for consumers are the spear appearance, such as shape and colour, as well as organoleptic qualities such as texture and flavour (Siomos, 2018). During post-harvest storage, spears can change in consistency, with the spears hardening due to lignification (Rodríguez et al., 2004; Anastasiadi et al., 2020). Loss of colour due to chlorophyll breakdown is also a problem, activated as a result of postharvest senescence (Mastropasqua et al., 2016). Vitamin C is also lost during post-harvest storage, as is the case for other fresh produce such as salads (Spadafora et al., 2016). A key limitation to asparagus shelf-life in the UK is, furthermore, the development of tip breakdown. This develops sporadically during chilled storage and is first detected as an unpleasant odour. The development of the aroma affects the whole punnet of spears resulting in substantial waste. Morphologically, tip breakdown is seen as water-soaked bracts and areas of internal browning at the tip of the spear (Fig. 1) likely caused by localised cell death. The affected areas then often develop microbial colonisation by pathogens such as *Fusarium* spp. (Lallu et al., 2000). Despite extensive research, the primary cause of tip breakdown has not been identified although links to sugar and abscisic acid (ABA) have been made (Anastasiadi et al., 2020). High ABA was linked to low sugar content and reduced shelf-life. The susceptibility to tip rot varies across cultivars: for example, warmer climate cultivars such as 'Jaleo' and 'Early California' appear to be less susceptible to tip breakdown than cooler climate cultivars such as 'Aspalim' and 'Gijnlim' (Anastasiadi et al., 2020 and commercial data, not shown). Indeed, the development of tip breakdown in the UK is more prevalent towards the end of the season when the weather is warmer and the asparagus is faster growing (Lill et al., 1996; personal communication, 2021). This led to the hypothesis that cell expansion may be accelerated resulting in larger, weaker cells more susceptible to damage.



Figure 1. Asparagus spear affected by tip breakdown. Areas of internal browning are indicated by arrows.

Transcriptome sequencing (RNASeq) is a powerful method for revealing metabolic pathways that may be involved in the development of post harvest syndromes (e.g., bent neck in rose, Lear et al., 2022; fungal spoilage in strawberries, Xiong et al., 2018) and potential treatments to reduce symptoms (Godana et al., 2022).

To investigate the cellular mechanisms of tip breakdown, we assessed cell size to establish whether it differed across cultivars or growth conditions and applied transcriptome analysis to compare healthy and affected spears.

MATERIALS AND METHODS

Plant material

Asparagus crowns of cv. Aspalim and cv. Early California were transplanted from the field, and grown under controlled conditions at Cranfield University with a day temperature of 18 or 28 °C and a night temperature of 8 or 14 °C. As spears developed, they were harvested (maximum height 27 cm), further trimmed to 16 cm to simulate commercial practice, and stored at 7 °C in the dark for 14 days either at Cranfield University, or following chilled transport at Cardiff University. Tip breakdown was assessed visually and scored (Table 1).

Table 1: External and Internal symptoms of tip breakdown in asparagus spears.

Tip breakdown stage	External symptoms	Internal symptoms
1	slight softening of very top of the tip	slight darkening in one small area
2	slight darkening & softening of the tip	slight darkening in a large / multiple areas
3	softening & darkening with slight watery tips but no tip disintegration	darkening and watery area(s)
4	tip disintegration	darkening/watery areas and slight disintegration
5	severe tip disintegration	severe tip disintegration

RNA extraction and transcriptome analysis

The top 2 cm of halved asparagus spears was freeze dried, and stored at -80 °C until used. RNA was extracted from 100 mg of the asparagus tissue by grinding under liquid nitrogen to a fine powder with a mortar and pestle followed by extraction using TRI reagent according to the manufacturer's instructions (Sigma Aldrich, Dorset, UK).

Following quality control of the RNA, it was sent to Novogene who performed the RNAseq analysis using an Illumina platform. Sequences were checked for base quality, low quality reads and adapters were removed and aligned to the Asparagus genome sequence (Harkness et al., 2017). Differential expression analysis used DESeq and expressed as log2 fold change (log2FC) and a threshold of p adj. <0.05 was applied to identify differentially expressed genes (DEGs).

Cellular analysis

The top 4 cm of asparagus spears of both cultivars were halved using a sharp scalpel and immediately immersed in fixative (formalin or paraformaldehyde) at harvest or after storage. Spears were embedded in paraffin wax and sectioned using a microtome. Slides were stained with heamotoxylin and eosin and cells visualized using a light microscope. Image J was used to measure their area. Smaller cells which might be incomplete or tangentially sectioned were removed from the calculation and data represent means of the largest 25% of cells (n>100) from two replicate spears. Statistical differences were assessed using a mixed effects model.

RESULTS

1. Cellular analysis

A comparison of cell size across healthy spears of two varieties contrasting in their susceptibility to tip breakdown and grown at a higher and lower temperature regime revealed no significant differences in cell size either due to growth temperature or due to the variety (Fig. 2).

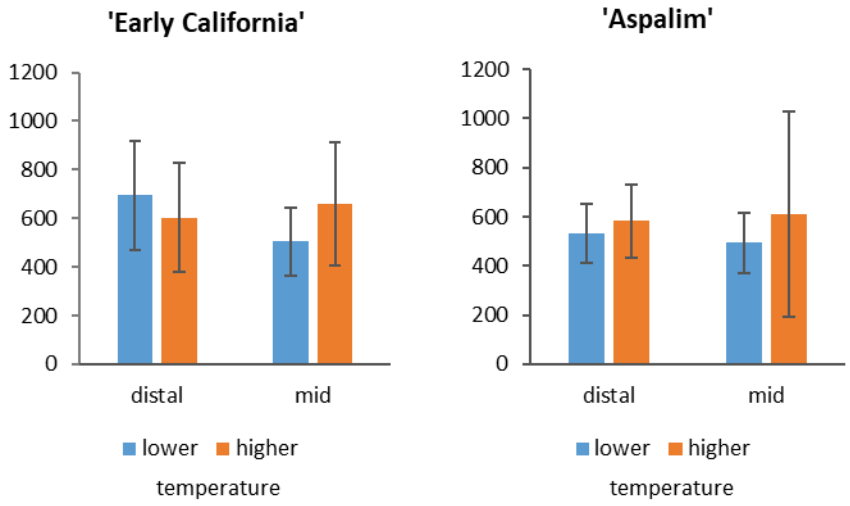


Figure 2: Mean cell area in the central region of asparagus tips of cv. Early California and cv. Aspalim grown at lower (8 °C night and 18 °C day) or higher (14 °C night and 28 °C day) temperature and stored at 7 °C for 14 days, (mean ± SE, n>100, the largest 25% of cells from two independent spears).

2. Transcriptome analysis

Transcriptome analysis was performed using RNA extracted from healthy spears, and those showing stage 2 of tip break down (Table 1) from cv. 'Aspalim', which is more susceptible to tip breakdown. All spears were collected from asparagus grown at the higher temperature regime (see Methods) and stored for 14 days at 7 °C. A total of 192 M raw reads (mean of 64.2M reads per replicate) were recovered from the three biological replicates representing tip breakdown, and 203 M reads for the healthy spears (mean of 67.9M reads per replicate). For sequences derived from spears with tip breakdown, 84.9% of reads, and for sequences from healthy spears, 83.7%, mapped to the Asparagus genome sequence.

Over 86% of sequences mapped to exons. A total of 892 genes were DEGs (Fig. 3) with more genes up-regulated in the spears showing tip breakdown compared to healthy spears.

GO enrichment analysis was used to investigate the functional categorization of the differentially expressed genes (Fig. 4). The largest number of DEGs were assigned to DNA binding and transcription regulation, and another group were related to transferase activity. Other categories of GTPase activity, oxidoreductases as well as heam and iron binding functions were also represented.

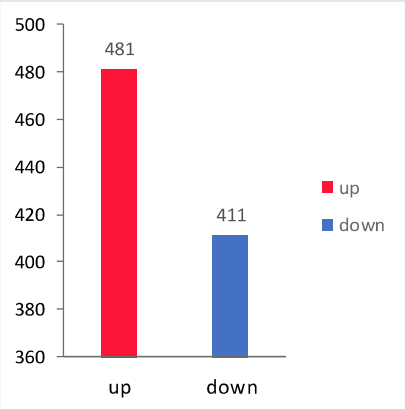


Figure 3: up and down differentially expressed genes (DEGs) between asparagus cv. Aspalim spears displaying tip breakdown and healthy spears, both grown at 14 °C night and 28 °C day and stored at 7 °C for 14 days (p adj <0.05).

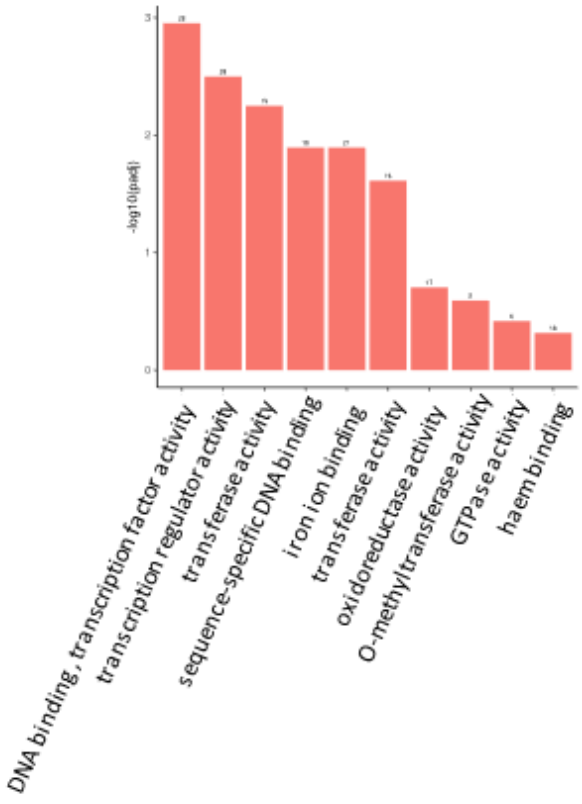


Figure 4: GO enrichment analysis of DEGs between asparagus cv. Aspalim spears displaying tip breakdown and healthy spears, both grown at 14 °C night and 28 °C day and stored at 7 °C for 14 days showing the GO category "molecular function"

DISCUSSION

An examination of cell size across the two varieties at the two growth temperatures did not reveal any significant differences in cell size. Fast growth of the spear could be due to rapid cell elongation resulting in larger cells. Stem growth is driven by a combination of cell division and cell elongation (Kutschera and Niklas , 2013). Moreover, environmental stress such as drought leading to reduced stem elongation, also results in reduced cell size (Litvin et al., 2016). The finding that cell size was not altered implies an increase in the rate of cell division at the apical meristem. Application of GA increases stem internode cell number (Yang, 1996) in *Pisum sativum* but did not change the rate of cell division in the shoot apical meristem (Daykin et al, 1997) and the authors concluded that the increased number of cells between internodes was due to local cell division. Hence further work could be directed in asparagus to assess both internode cell number and meristem cell division to understand better the mechanism driving the more rapid spear elongation at warmer temperatures.

A key aim of this work was also to investigate any changes in gene expression occurring in asparagus spears affected by tip breakdown using RNAseq. The publication of the asparagus genome (Harkess et al., 2017) has greatly facilitated this approach, together with advances in next generation sequencing and bioinformatic analysis. At stage 2 of tip breakdown the damage is still fairly localized. Moreover, the spear tip is a complex organ made up of a large number of cell types at different stages of cell division and expansion. It was therefore perhaps surprising that substantial number of genes were found that changed significantly in expression between healthy spears and those affected by tip breakdown symptoms. The large number of up-regulated genes suggests that the process is active and not a passive or necrotic cell death. Several distinct types of cell death are recognized in plant cells elicited by pathogens (the hypersensitive response), development or stress-induced senescence. These are all considered active processes and distinct from very rapid necrotic cell death that can be elicited for example by trauma such as sudden excessive heat or toxic chemical exposure (Van Breusegem and Dat, 2006). Non-necrotic plant cell death is associated with changes in gene expression (Valandro et al., 2020), hence the damage seen in tip breakdown is likely to fit one of these cell death mechanisms.

A preliminary analysis of the types of genes that change in expression during tip breakdown reveals a high proportion of genes associated with transcriptional regulation. These will be interesting to investigate further to assess whether they perform a regulatory function. Specific classes of transcription factors are associated with senescence and stress responses, such as the NAC and WRKY transcription factor family genes (Nakashima et al., 2012; Tsuda and Somssich, 2015) and it will be interesting to assess whether these are also upregulated in this system. Several metabolism-related genes were also altered in expression including transferases, oxidoreductases and O-methyltransferases. Further investigation of these genes may provide clues to the mechanisms underlying the development and spread of the tip breakdown and offer potential for treatments or breeding targets for improving resilience in the more susceptible varieties.

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