Understanding the role of phytohormones in controlling heat stress tolerance during pollen development in wheat

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1. Introduction

Cereal crops are highly **vulnerable** to **heat stress**. Wheat exposed to 3 days at 30°C during pollen development displays a **35% reduction in grain set**[1], consequently a rise in global temperatures by 1°C is expected to result in a **6% loss in global wheat yield**[2]. Currently, rising global temperatures have caused annual losses of around **$5 billion** in wheat, maize and barley[3]. With global temperatures predicted to continually increase, heat stress induced loss of yield is set to become an increasing problem. In wheat, heat stress induces early **tapetum degradation**, **pollen disorientation** and **disrupts meiosis and mitosis** in developing pollen (fig.1)[4], resulting in the production of **inviable pollen** and **male sterility.** As wheat is predominantly self fertilising, depletion of viable pollen reduces grain set and yield.

The phytohormones, **auxin**, **jasmonate** and **ethylene** have an important role in controlling **pollen development** and have been implicated to play a role in regulating **heat stress tolerance**. To tackle the problem of heat stress, a greater understanding of the roles of these phytohormones is crucial.

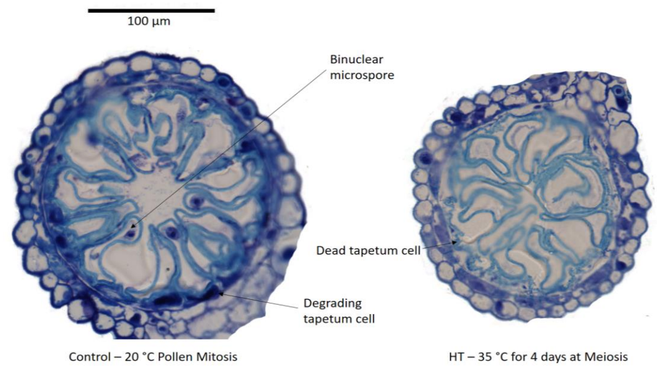

Fig.1: A cross section of wheat anthers from a control and a heat stressed plant exposed for 4d at 35 °C from microsporocyte meiosis.

2. Aims

To further our understanding of the role of these phytohormones in pollen development and heat stress tolerance, this project aims to:

- Generate wheat hormone signaling mutants using **TILLING** technology and characterise their response to heat stress.
- Identify transcriptional and hormonal changes in developing anthers during normal conditions and in response to heat stress.
- Establish how application of synthetic signaling molecules affects pollen development in response to heat stress.

3. Auxin mutants

Auxin is a plant hormone involved in mitigating abiotic stress. Aux/IAAs are **transcription repressors** of auxin responsive genes. Auxin recognises Aux/IAAs through the conserved **GWPPV domain** and induces their degradation. Missense mutations in this conserved domain results in auxin-insensitivity[5]. **Nine** potential auxin-insensitive mutants were characterised from the wheat TILLING population (fig.2).

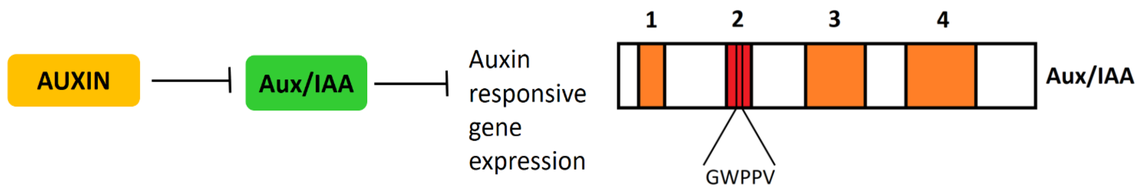

Fig.2: The auxin signalling pathway and the domain structure of Aux/IAAs.

4. Jasmonate mutants

Jasmonate is plant hormone involved in reducing abiotic stress. JAZ proteins are **repressors** of transcription factors involved in improving abiotic stress tolerance. Jasmonate induces destruction of JAZ proteins via COI1 mediated degradation. **Alternative splicing** of the jas intron, which divides the jas motif, results in **truncated** versions of JAZ proteins that are **resistant to degration**[6] (fig. 3). **Three** potential jasmonate-insensitive mutants have been found by identifying splice-site mutations in the C-terminal jas intron.

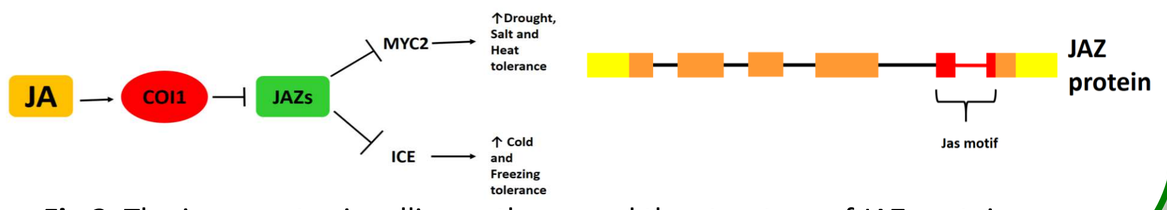

Fig.3: The jasmonate signalling pathway and the structure of JAZ proteins.

5. Ethylene mutants

Ethylene is a gaseous hormone released in response to abiotic stress, and is associated with yield loss in crops. Missense mutations in **conserved residues** of ethylene receptors lead to an ethylene-insensitive phenotype (fig. 4)[7]. **Five** potential ethylene-insensitive TILLING mutants have been characterised, based on mutations which confer amino acid changes in these conserved residues.

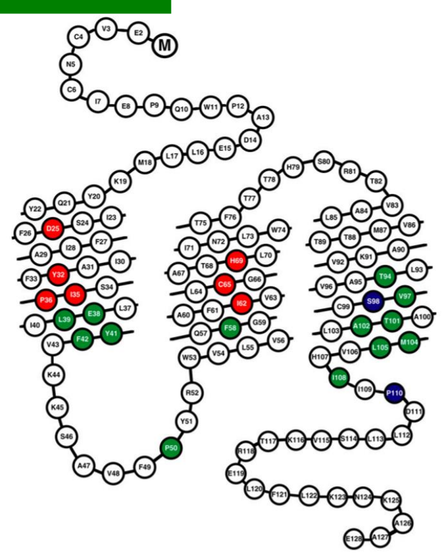

Fig.4: The transmembrane domain of AtETR1[7]. Red aa are ethylene binding sites and green are involved in the ethylene response.

6. Use of the Ampha Flow Cytometer

The Ampha Z32 Impedance Flow Cytometer provides the opportunity to use a high throughput method of staging wheat anthers based on the developmental stage of the pollen. It also provides a means of measuring defects in pollen development in the field as a forward genetic screen or directly investigating the impacts of hormone insensitivity on the development of the pollen.

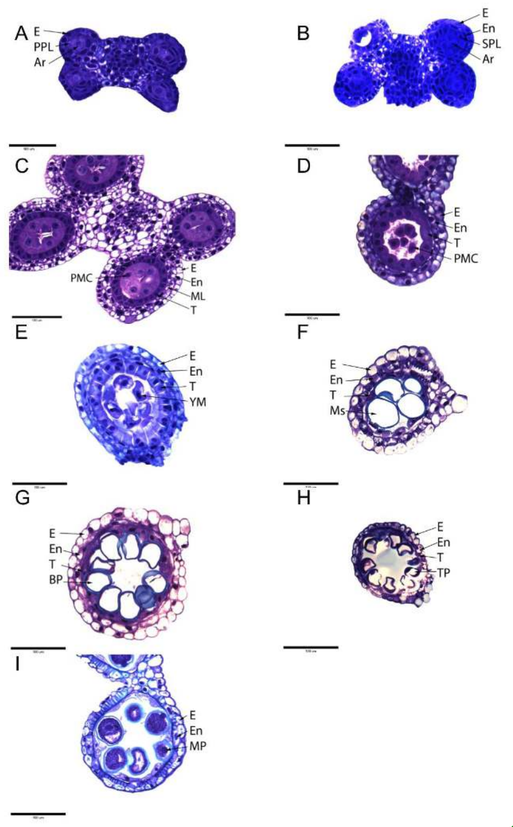

Fig. 5. Nine distinct stages of wheat anther development (scale bar= 100µm).

7. Conclusion

Having these signalling mutants is a novel resource to be used in wheat research. The use of these mutants is not only limited to investigations into pollen developmental and heat stress tolerance, but also a variety of other forms of stress.

Future work using the Flow Cytometer

- Investigate the impact of heat stress on pollen viability of the signalling mutant plants, both in normal and heat stress conditions.
- Investigate changes in hormone and transcription levels at different stages of anther development and during heat stress.

References

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