Microscopic Analysis of Intrinsic Puroindoline Fluorescence

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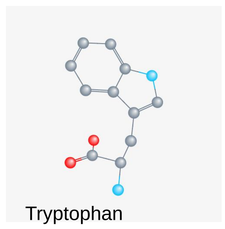
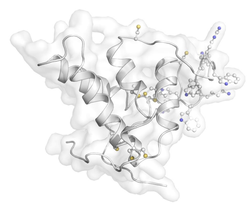
Introduction

- Cysteine and tryptophan (Trp) are the rarest occurring amino acids in proteins[1]. The aromatic structure of the indole rings of Trp gives the amino acid intrinsic fluorescence.
- Puroindolines (PIN) are starch granule (SG) associated proteins that confer endosperm hardness in wheat[2]. PIN A contains five Trp in a seven residue stretch as well as a C-terminal Trp residue.
- Soft white winter wheat (SWWW) have a rich concentration of PIN[2]. Is it possible to view the PIN localized to the SG surface utilizing Trp autofluorescence?



Tryptophan



Animation courtesy of Prof. Corrie daCosta.

Puroindoline amino acid sequence

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  1 mkalfligll amvastafaq ysevvgsydv
 31 aggggaqqcp vetklnscrn ylldrcstmk
 61 dfpvtwrwwk wwkggcqell geccsrlgqm
 91 ppqcrcniiq gsiqgdlggi fgfqrdrask
121 viqeaknlpp rcnqgppcni pgtigyyw
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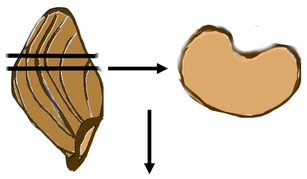
Methods

Objective

To image PIN fluorescence without the use of antibodies and other fluorescence markers which produce artefact(s).

AC Augusta (SWWW), #7 Durum wheat and M202 transgenic rice (97-1) were grains used in this experiment. Rice and wheat seed endosperm were both hand cut and sectioned on a Microm™ cryostat. Hand cut cross-sections were imaged on a Carl Zeiss AxioImager M2 DIC Microscope.



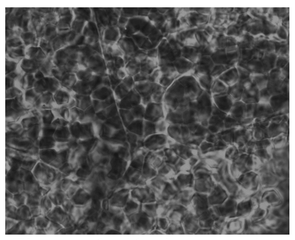
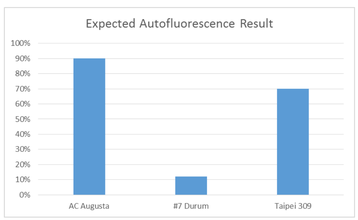
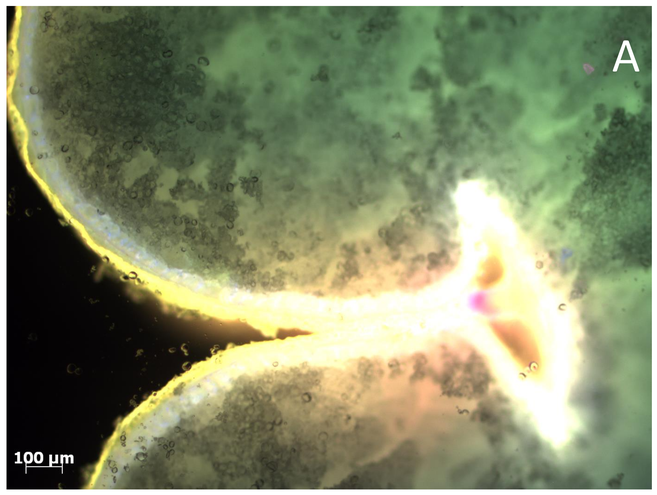
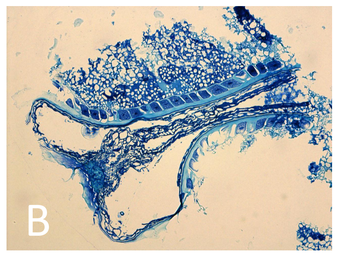


Figure 1. Kaybonnet rice cross-section at 100X magnification. (source C. Melnyk)



Results & Discussion





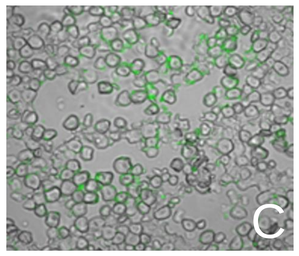


Figure 2. (A) AxioVision image of a hand cut Augusta cross-section. (B) Augusta cross-section stained with Toluidine Blue. Light microscopy. (C) Chinese Spring soft wheat, showing immunofluorescence around the SG. Images B and C were taken by Charles Melnyk.

- Previous microscopy studies of PIN have used antibodies and fluorochromes to induce fluorescence[3]. Introduction of exogenous fluorescent bodies are likely to produce artefacts.
- Autofluorescence of PIN has yet to be imaged *in situ*. Hand cut cross-sections are too thick for reflection fluorescence microscopy, but, thin cryostat sections of approximately 8µm crumble when sectioned.
- Moving forward: Resin embedding of the grain samples may be required before cutting the friable grain in a cryostat.

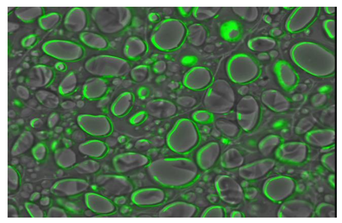


Figure 3. Example of an immunofluorescence endosperm section that likely shows more artefact fluorescence that factual PIN localization. (source C. Melnyk)

Conclusions

- Comparison of fluorescence in cross-sections of *Triticum durum* (PIN-null), soft *Triticum aestivum* (PIN rich), and 97-1 transgenic rice (PIN+) grains may confirm the association between puroindoline and the SG surface proposed from previous studies.
- Further investigations are required to determine which embedding technique would best suit autofluorescence microscopy analysis. Good embedding material should not promote fluorescence in untargeted proteins in the wheat endosperm.
- Confirmation of PIN and SG may validate the fusion of PIN to proteins of interest in rice plant expression of recombinant proteins (rtP)[4]. Production of rtP in transgenic rice grains is poised to reduce the cost of downstream processing for manufacture of rtP in a cleaner process with less water waste.

Bibliography

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4. Krishnamurthy K and Giroux M. (2000) Expression of wheat puroindoline genes in transgenic rice enhances grain softness. *Nature Biotechnology*. 19: 162-166.

Acknowledgements

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