Microscopic Analysis of Intrinsic Puroindoline Fluorescence
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Introduction

- Cysteine and tryptophan (Trp) are the rarest occurring amino acids in proteins. 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. 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 high concentration of PIN. Is it possible to view the PIN localized to the SG surface utilizing Trp autofluorescence?

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)

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.

Results & Discussion

- Previous microscopy studies of PIN have used antibodies and fluorochromes to induce fluorescence. 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). 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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