Discovering site of action of herbicide Indaziflam

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INTRODUCTION

- Indaziflam is a pre-emergence, contact herbicide
- It is mainly used in turfgrass, orchard, rangeland and forestry systems for control of annual weeds
- Classified as cellulose biosynthesis inhibitor
- Acts on actively dividing cells

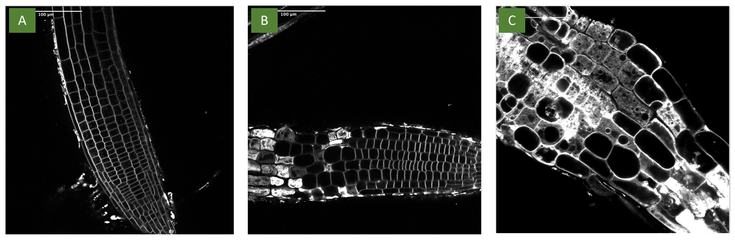

Figure 1 –All images are taken using confocal microscope 40X objective
A: 4 days old *Arabidopsis thaliana* root stained with FM4-64
B: 4 days old *Arabidopsis thaliana* root treated with 8000 nM indaziflam and stained with FM4-64
C: 4 days old *Arabidopsis thaliana* root treated with 100 nM isoxaben and stained with FM4-64

	Isoxaben	Indaziflam
Broad spectrum activity	No	Yes, monocots and dicots
Cellulose synthase A	Complete inhibition, decreased CESA in plasma membrane	Reduced velocity, increased CESA in plasma membrane
Arabidopsis mutant resistant to traditional CBI	Yes	No

Table 1 – Comparison between the indaziflam and Isoxaben. Isoxaben is a know cellulose synthase A inhibitor. Indaziflam though classified as cellulose biosynthesis inhibitor, shows some inconsistency. This suggest that it may have different site-of-action.

HYPOTHESIS

- Indaziflam is not a true Cellulose Synthase A inhibitor, it inhibits CME which manifests with CBI like symptomology

OBJECTIVES

- To test unintended effects of indaziflam on non-plant organisms
- To find site of action of herbicide indaziflam
- To find if indaziflam acts as an endocytosis inhibitor

MATERIALS AND METHODS

- **Experiment 1 – Effect of indaziflam on fungi growth**
  - Agar plates with different indaziflam concentrations were made
  - Trichoderma and Rhizoctonia fungi were used
  - Growth was noticed over a period of 2 weeks
- **Experiment 2 – Quantitative effect of indaziflam on yeast**
  - Quantitative measurement of effect of indaziflam on yeast
  - Yeast strain yMK839 was used
  - 100 ml YPD media was used for each treatment
  - 6 different concentrations of indaziflam was used
  - OD600 reading were taken at 3 hrs. interval for 24 hrs
- **Experiment 3 – Co-IP precipitation assay**
  - Indaziflam was conjugated to NHS FlexiBind magnetic beads
  - *Arabidopsis Thaliana* and *Oryza sativa* root protein extract was used
  - The supernatant was passed through the beads
  - Liquid chromatography with tandem mass spectrometric protein analysis was done
- **Experiment 4 - Measuring endocytosis using confocal microscopy**
  - Confocal microscopy was performed on Olympus fluoview spectral FV1000
  - DMSO control, Indaziflam, Isoxaben and Dynasore was used
  - 3 time points – 5 min, 20 min and 40 min was selected
  - FM4-64 Dye was used for fluorescent purposes
  - Quantification was done using ImageJ

RESULTS

Experiment 1 - Effect of indaziflam on fungal growth

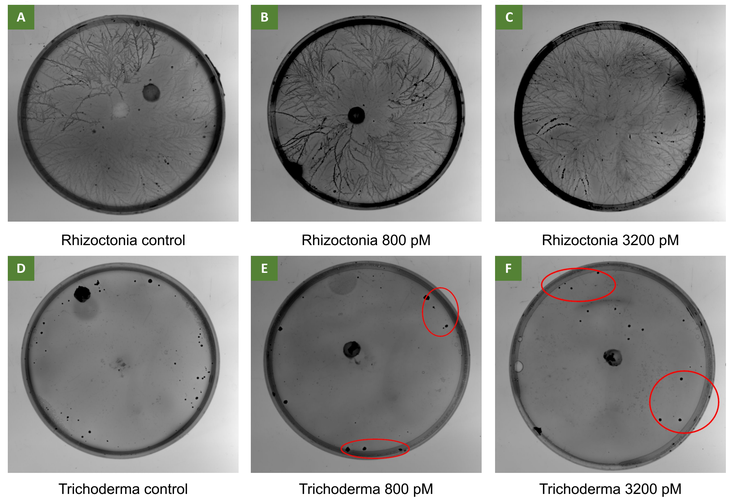

Figure 2 – Effect of indaziflam on Rhizoctonia and Trichoderma fungal growth. LD50 of indaziflam on plants in 800 pM. Maximum concentration of 3200 pM was also tested. A: Rhizoctonia with no indaziflam. B: Rhizoctonia with 800 pM concentration C: Rhizoctonia with 3200 pM concentration D: Trichoderma with no indaziflam. E: Trichoderma with 800 pM concentration F: Trichoderma with 3200 pM concentration. Red circles indicate spore formation.

Experiment 2- Quantitative Effect of indaziflam on yeast

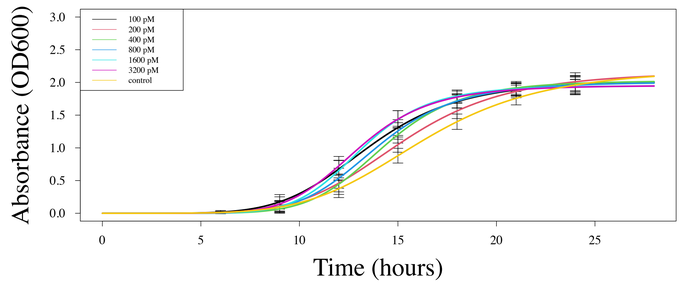

Figure 3 – Effect of indaziflam on the growth of yeast measured over 3 hrs interval. Optical Density 600 (OD600) was measured using spectrophotometer. 3 replicates was done, with lowest concentration of indaziflam being 100 pM and highest being 3200 pM.

Experiment 3- Co-IP precipitation assay

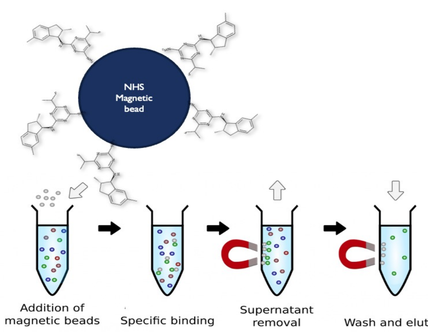

Figure 4 – Protocol of Co-IP precipitation assay. Indaziflam binds to NHS magnetic beads and is suspended in the elution mixture. The root lysate extracted from *Arabidopsis thaliana* and *Oryza sativa* is passed through it. The proteins that get attached to indaziflam is purified and sequenced using LC-MS/MS. Homologous proteins were identified, and a list of 33 proteins in *O. sativa* and 50 proteins in *A. thaliana* were generated.

		A. thaliana	O. sativa
Group no.	Protein Group	Enrichment (indaziflam/control)*	Enrichment (indaziflam/control)*
1	Endocytosis/Exocytosis	111.61/14.12	172.8/44.56
2	Energy supply	76.42/20.97	77.45/0
3	Protein translation	47.1/11.64	34.21/7.23
4	Glycosyltransferases	57.21/11.11	38.47/2.48
5	Non-target site herbicide resistance (NTSR	44.4/6.04	39.76/4.81
6	Phosphorylation	55.74/20.91	7.74/0
7	Miscellaneous functions	84.397/26.81	105.71/45.23

Table 2 – List of the proteins extracted from the precipitation assay. They are grouped based on their function. Most enriched proteins were involved in endocytosis/exocytosis, specifically Clathrin-mediated endocytosis.

RESULTS

Experiment 4- Measuring endocytosis using confocal microscopy

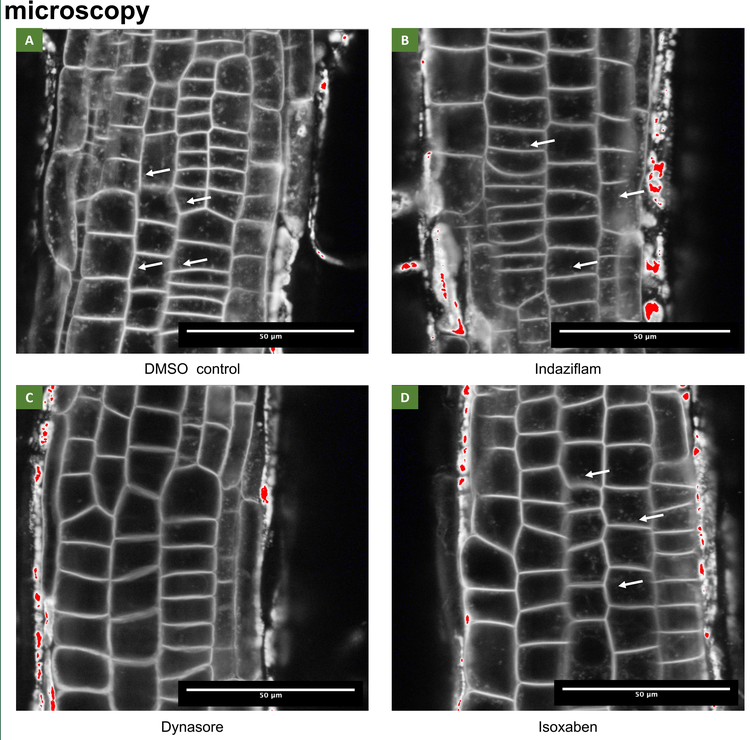

Figure 4 – The images are enhanced using enhance contrast function of ImageJ. The saturated pixel values are 0.35% and equalize histogram selection was used. The quantification was done on raw images with no post- acquisition enhancements. A: 4 days old *Arabidopsis thaliana* root stained with FM4-64, treated with DMSO 0.001% concentration as a control. B: 4 days old *Arabidopsis thaliana* root treated with 8000 nM indaziflam, in DMSO 0.001% for 2 hrs. and stained with FM4-64. C: 4 days old *Arabidopsis thaliana* root treated with 80 uM Dynasore, in DMSO 0.001% for 3 hrs. and stained with FM4-64. D: 4 days old *Arabidopsis thaliana* root treated with 100 nM isoxaben, in DMSO 0.001% for 2 hrs. and stained with FM4-64. All images are taken using confocal microscope 40X objective.

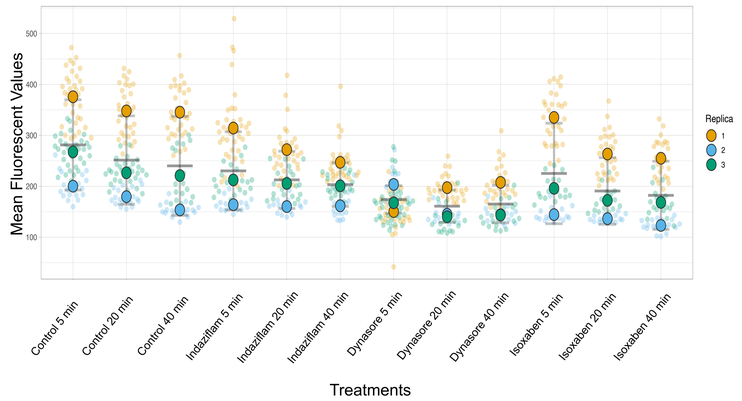

Table 4 - Mean fluorescent values of various treatments measure at 5min, 20min and 40 min time-points. 3 replicates are being represented. All conditions are consistent between replicates.

CONCLUSIONS

- The site of action of indaziflam is specific to plants
- Preliminary data suggests site-of-action is in endocytosis/exocytosis pathway
- Cytology data does not show any decrease in endocytosis
- Future research will focus on how indaziflam effects auxin transport in root tips

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