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Does an auxin-adjuvant urea derivative interfere with the vascular pattern formation?

Preliminary Results

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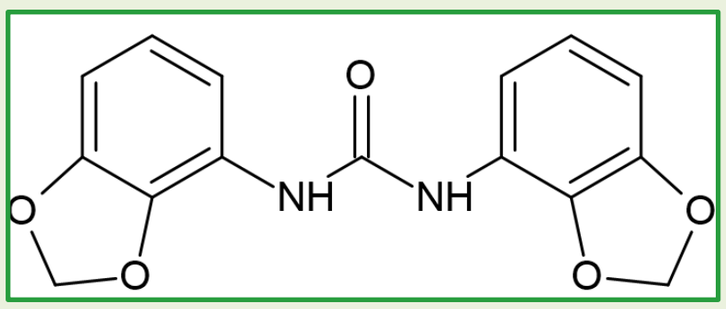
INTRODUCTION

VASCULAR PATTERN FORMATION

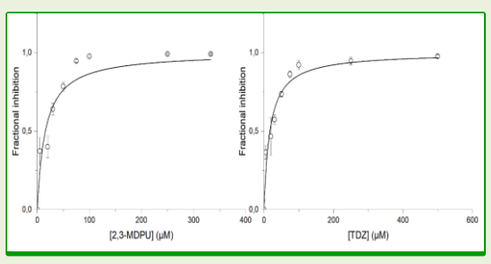
- The vascular system is composed of a hierarchical network of interconnected veins; it is essential for land plants because it provides long-distance transport of water, nutrients, and signaling molecules
- Vascular pattern formation is a self-organizing process regulated by auxin
- Tissues differentiate in regions where high auxin concentration is present → supplementation of exogenous auxin or hormone transport inhibitors change venation pattern
- Auxin binds Auxin Binding Protein (ABP1): this binding combined with directional transport of the hormone, acts to form the vascular system

UREA DERIVATIVE: N,N'- bis-(2,3-methylenedioxyphenyl)urea

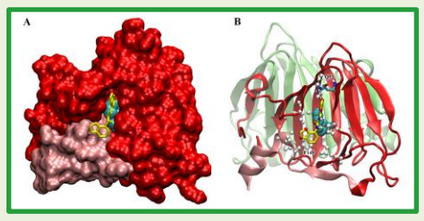


2,3-MDPU

- It enhances adventitious root formation in Gymnosperms and Angiosperms
- It does not interfere with the polar auxin transport
- It inhibits cytokinin oxidase/dehydrogenase (CKX) **(1) (Ricci et al, 2023)**
- It interacts with the apoplastic portion of the auxin receptor ABP1 **(2) (Ricci et al,2023)**



(1)



(2)

AIM

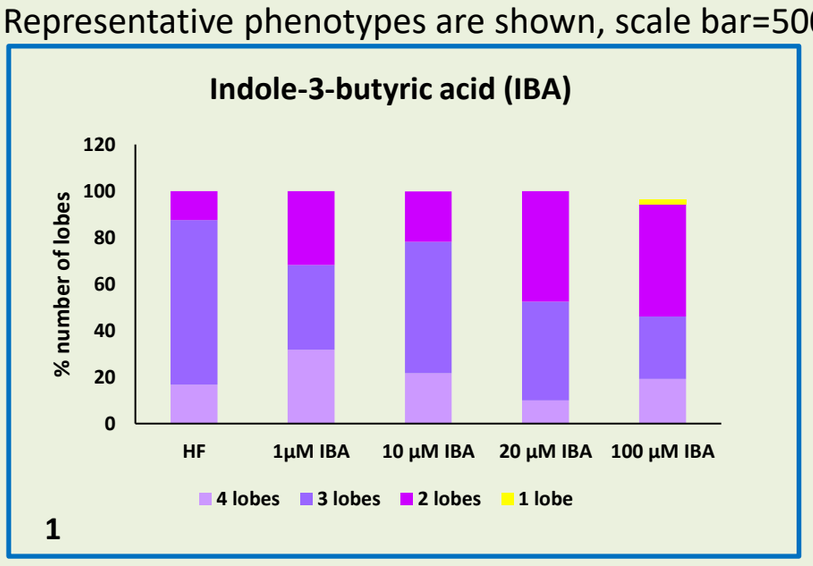
The aim of the study is to evaluate whether urea derivatives can interfere with vascular pattern formation and how they can do so.

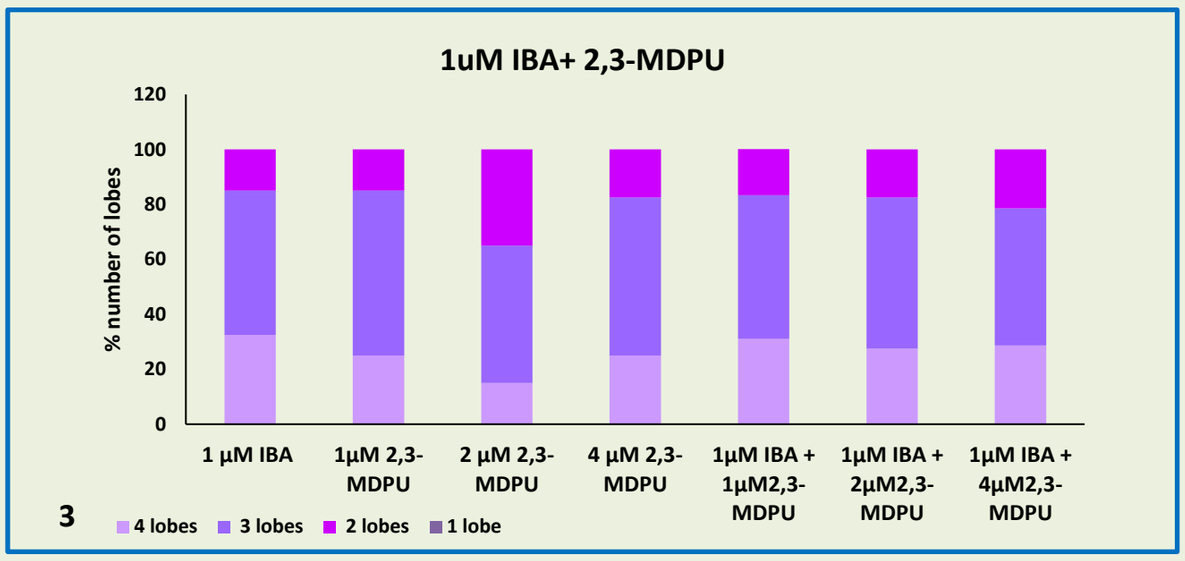
METHODS

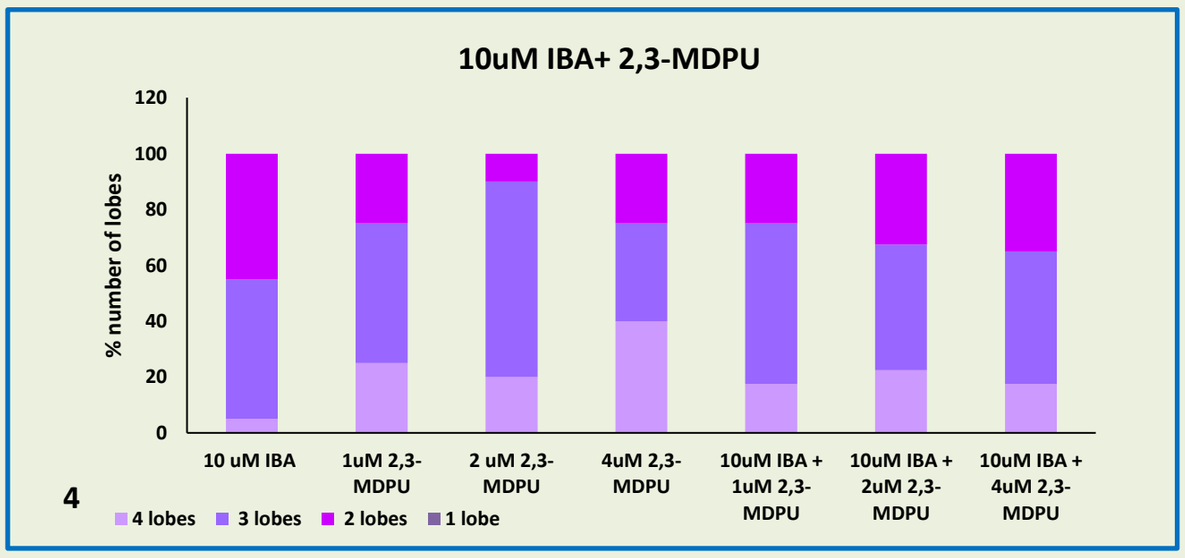
- *Arabidopsis thaliana* ecotype Columbia (Col-0) seeds, were sterilized in commercial bleach for 10 minutes and washed five times in sterile distilled water
- They were placed on culture medium (1\4 strength MS salt plus 1% (w\v) sucrose, 0.8% (w/v) Phytoagar (Duchefa), 5.8 pH) supplemented with 1 µM, 10µM, 20 µM and 100 µM IBA or 1 µM, 2µM and 4µM 2,3 MDPU or IBA plus 2,3- MDPU
- After a cold treatment at 4°C for 3 days in the darkness, the plates, containing 20 seeds each, were incubated in a growth chamber at 23±1°C at a light intensity of 27 µmol m^{-2} s^{-1} under 16h photoperiod
- *Arabidopsis thaliana* cotyledons were taken 13 days after sowing and they were incubated in 70% ethanol for at least 48h (two passages 24h each)
- Observation of the morphology of the cotyledons under a light microscope Zeiss AxioLab 5, Axiocam 208 color

RESULTS & DISCUSSION

Analysis of cotyledons venation pattern. The frequency of the number of lobes depends on the supplemented compounds and is expressed as percentage of the total number of the cotyledons analyzed in each experimental condition. Representative phenotypes are shown, scale bar=500 µm







CONCLUSION AND FUTURE WORK

The complexity of vascular system, *i.e*. the number of lobes, depends on auxin. The number of lobes decreases as the exogenous auxin concentration increases (Fig 1). Work is in progress to better analyze the reduction of number of lobes in the presence of 2,3- MDPU (Fig. 2), while the results obtained in the simultaneous presence of IBA plus 2,3-MDPU have to be confirmed (Fig. 3 and 4). Then the expression of related genes involved in the formation of the vascular system, such as *PIN*, *VCC* and *MONOPTEROS* will be evaluated.

REFERENCES: Biedron et al.2018, Plant Cell Rep,37(9):1215-1229. Gelova et al.2021,Plant Science,303,pp110750. Ricci et al.2023, Plants, 12,3610