Dichlofenac altered root developmental processes of Arabidopsis via interfering with the hormonal activities of auxin

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Abstract

Diclofenac, a pharmaceutical product, is detected in various environmental matrices and regarded as potential pollutant worldwide. Increased irrigation of water in agriculture has facilitated accumulation of environmental diclofenac inside green fresh products, which potentially threatens non-target organisms including human. In current study, we demonstrated that diclofenac inhibited the growth and altered root developmental processes of plants in a similar but competitive way to auxin, a group of major phytohormones. Exogenously treated diclofenac caused oxidative stress in *Arabidopsis* seedlings. In developmental point of view, diclofenac modified the root system architecture such as primary and lateral roots. The effects of diclofenac on the root development of *Arabidopsis* were mediated through canonical auxin signaling pathways. Interestingly, when diclofenac and auxin were used in combination, diclofenac suppressed the activity of auxin. Conclusively, diclofenac acted as a weak agonist of auxin in the root development of *Arabidopsis*, but interfered with natural auxin activities via competitive association with auxin signaling factors. Such results suggested that diclofenac could potentially behave as an environmental hormone by disturbing the natural developmental processes of plants.

Materials and Methods

Chemicals and plant materials

- Chemical: Diclofenac (DCF), 2-[2-(2,6-dichloroanilino)phenyl]acetic acid
- Plant: *Arabidopsis* Col-0 ecotype, *arf7arf19* mutant, *DR:GUS* expressing transgenic line hydroponically in 0.5X Hoagland media
- Seedlings were grown either in hydroponic media or MS salt media

Monitoring growth and development under DCF + conditions

- Biomass, oxidative stress status based on DAB staining and [H_2O_2]
- Root system architecture (root morphology, primary and secondary root)

Interference of DCF with auxin

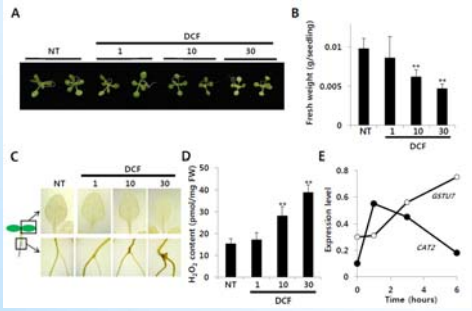
- GUS staining (driven by artificial auxin responsive promoter *DR5* activity)
- Side-by-side comparison of seedlings treated with DCF and/or auxin
- Monitoring responses of arf7arf19 mutant to DCF and/or auxin

Investigation of early molecular responses to DCF

- Selection of auxin responsive marker genes (*SAUR-AC1, GH3.1, LBD16 & 29*)
- Monitoring expression of the marker genes according to DCF exposure
- Examination in early time courses (0, 3, and 6 hrs after DCF treatment)

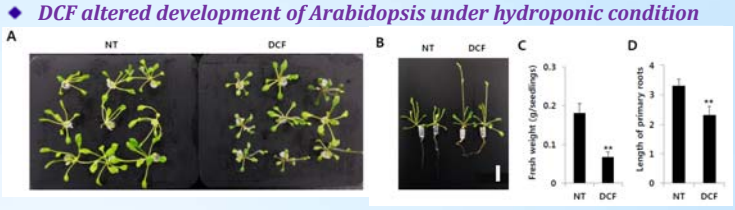
Results

DCF inhibited the growth of Arabidopsis



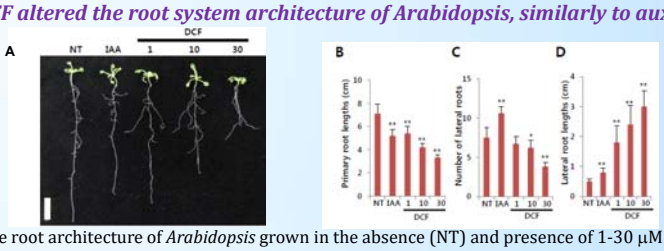
A. The morphology of *Arabidopsis* seedlings grown in the absence (NT) and presence of diclofenac (DCF μM). **B**. The average fresh weight of *Arabidopsis* seedlings **C**. DAB stained *Arabidopsis* leaves and roots. **D**. The average H_2O_2 contents measured in *Arabidopsis* seedlings. **E**. The relative expression level of marker genes for cellular oxidative stress in *Arabidopsis*.

DCF altered development of Arabidopsis under hydroponic condition



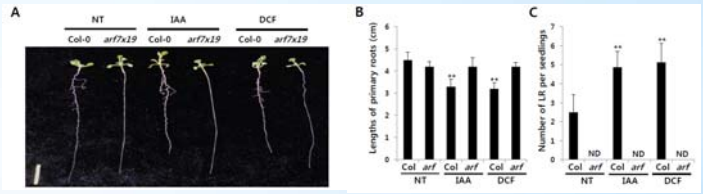
A. The morphology of *Arabidopsis* seedlings grown in the absence (NT) and presence of 10 μM DCF in hydroponic media. **B**. The leaves and roots of *Arabidopsis*. The average fresh weight (**C**) and primary root lengths (**D**) of *Arabidopsis* seedlings.

DCF altered the root system architecture of Arabidopsis, similarly to auxin



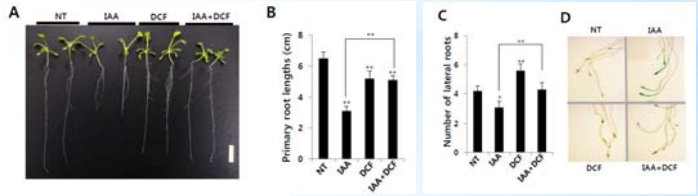
A. The root architecture of *Arabidopsis* grown in the absence (NT) and presence of 1-30 μM of DCF or IAA (50 nM), a natural form of auxin. The primary root length (**B**), number of lateral roots per seedling (**C**), and lateral root lengths in the maturation zone (**D**) of *Arabidopsis*.

DCF action was mediated via canonical auxin signaling pathways



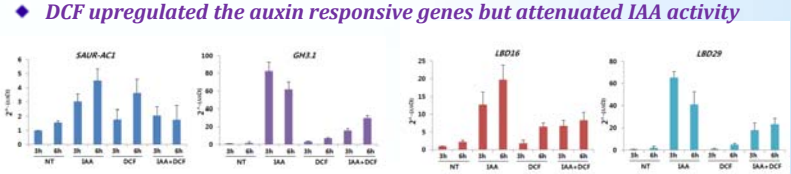
A. The architecture of *Arabidopsis* grown in the absence (NT), presence of 50 nM of IAA- or 10 μM DCF. The primary root length (**B**) and number of lateral roots per seedling (**C**) of *Arabidopsis*. *arf7arf9*: Arabidopsis mutants in which two major auxin signal regulators (ARF7 & ARF19) was abolished.

DCF suppressed the activity of exogenous auxin in Arabidopsis



A. The roots of *Arabidopsis* in the absence (NT), presence of 10 μM DCF, and/or 100 nM IAA. The primary root length (**B**) and number of lateral roots (**C**) of *Arabidopsis*. **D**. The GUS staining intensity in the roots of transgenic *Arabidopsis* seedlings harboring *DR5:GUS* , an auxin-responsive reporter. **E**. The absorbance of colorimetric products formed via GUS activity in *Arabidopsis* harboring *DR5:GUS* genes.

DCF upregulated the auxin responsive genes but attenuated IAA activity



The expression profiles of auxin responsive Arabidopsis genes according to trasient exposure to IAA (100 nM) and/or DCF (10 μM).

Conclusion

- Diclofenac (DCF) induced oxidative stresses in Arabidopsis seedlings
- *DCF altered the root system architecture of Arabidopsis seedlings in a similar fashion to phytohormone auxin*
- *Auxin-like behavior of DCF was mediated through the canonical auxin signaling*
- DCF suppressed the activity of exogenously treated auxin
- DCF acted as a weak agonist of auxin as well as a competitive inhibitor of auxin

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