Higher Education for Sustainable Food Production

4th Joint Meeting of Agriculture-Oriented PhD Programs

UniCT, UniFG and UniUD

Paluzza (UD), 3 – 7 October 2022

Chitosan nanoparticles for sustainable agriculture: interactions with leaf surface and protective effect on dsRNA as functionalizing agent

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An overview on the subject

Agriculture and environment: more sustainable practices are needed

Nanotechnology for plant disease and nutrition management

My topic of study: nanoparticles functionalized with bioagents

In detail …

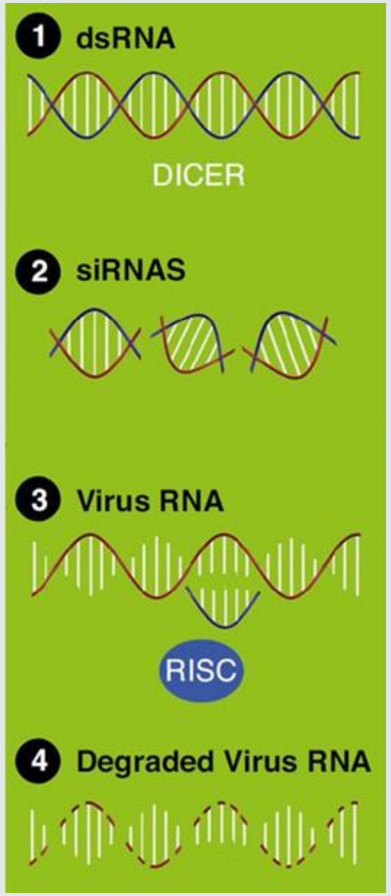
Chitosan nanoparticles (CHIT-NPs)

protective, carrier and biostimulant functions

Exogenous dsRNA

Against pathogen or weed

Induction of the RNA-interference (RNAi) mechanism



Mitter & Worrall, 2017

REFERENCES CHIT-NPS: Malerba & Cerana, 2016; Saharan & Pal, 2016; Kashyap *et al.*, 2015.
RNAi: Mitter & Worrall, 2017; Nerva *et al.*, 2020.

Aim of the project

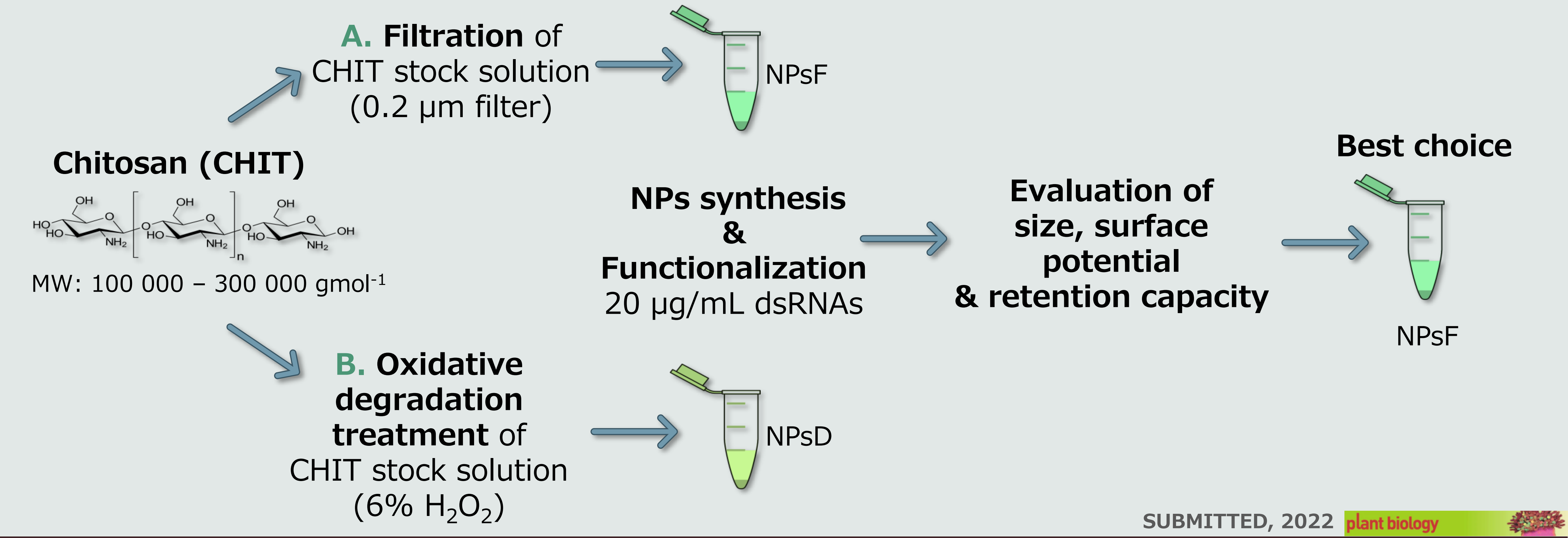
Evaluate whether the effect of dsRNA transported through chitosan NPs is improved compared to the application of naked dsRNA

Secondary purposes:

Biostimulation by CHIT and functionalizing agents

Interaction with plant surfaces and tissue entering pathways

Purification and characterization of other nanomaterials



1st year activities: definition of the best protocol for synthesis and functionalization of nanoparticles

2nd year main tasks

1. Evaluation of the behavior of NPs after application on leaves

I. ***Nicotiana benthamiana*** **Domin (as a model plant)**

II. *Amaranthus hybridus* L. (a common weed) – preliminary tests

2. Assessment of the ability of NPs to protect dsRNAs from degradation

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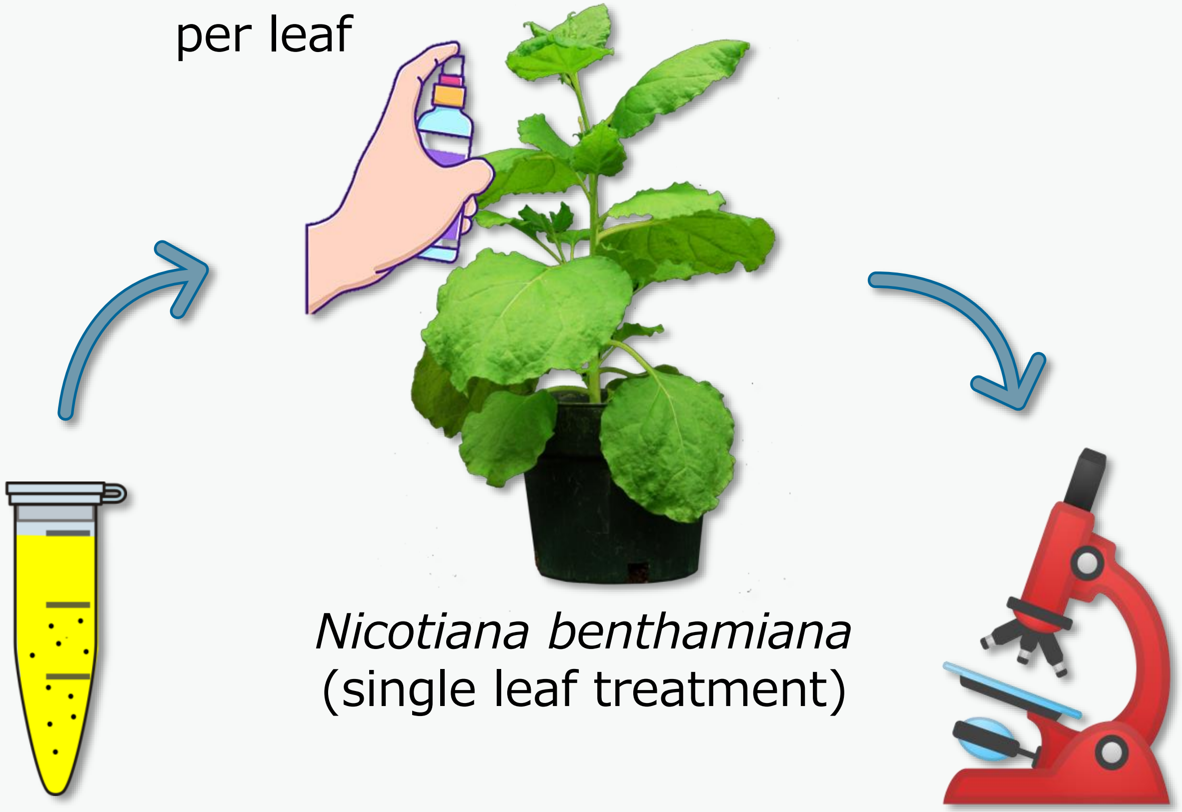
Total RNA from a transformed *E. coli* strain able to synthetize the dsRNAs of Green Fluorescent Protein (***GFP*-dsRNA**) (Nerva *et al.*, 2020)

1. Evaluation of the behavior of NPs after application on leaves

Methods

0.5 mL
per leaf

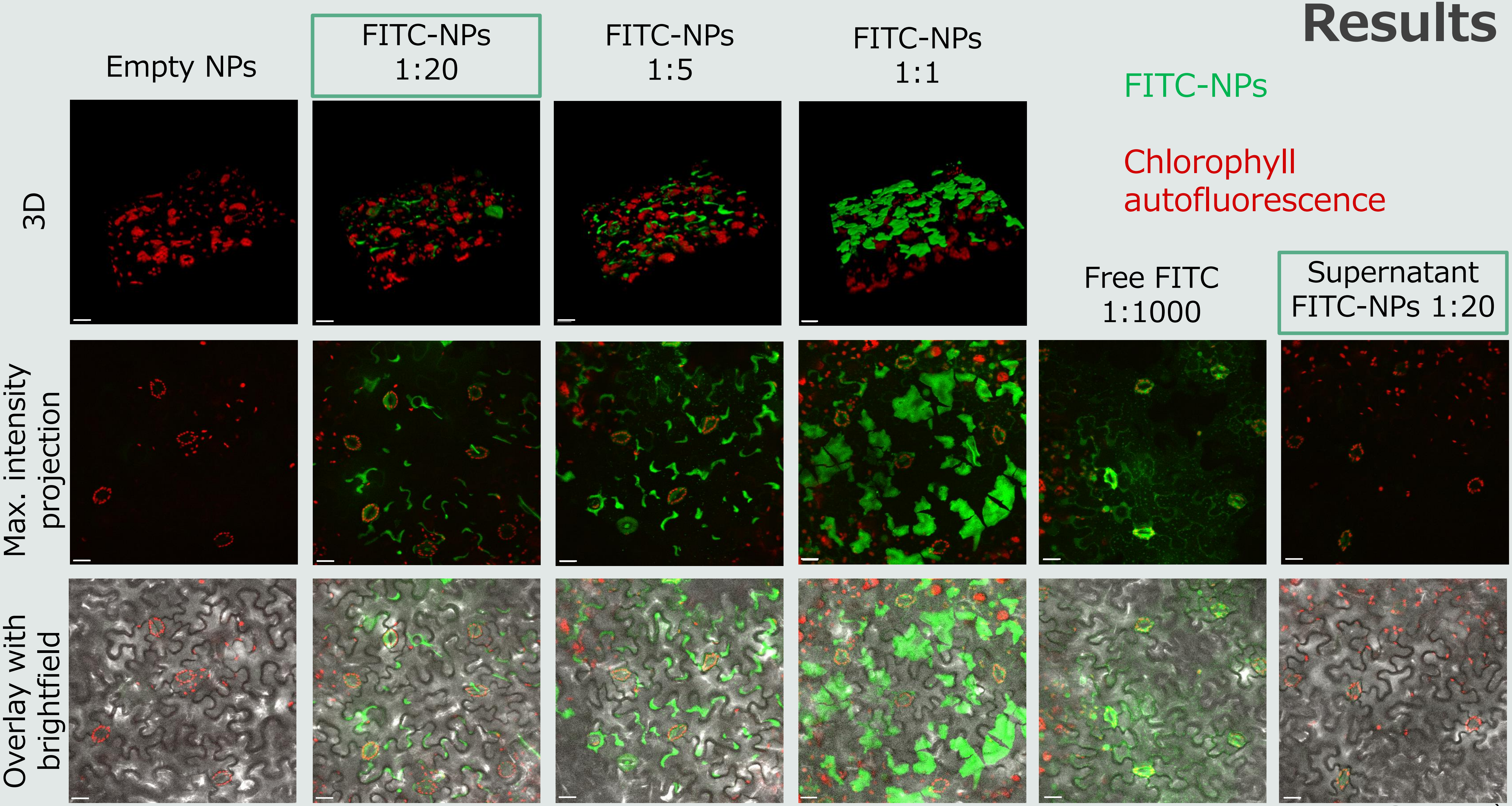


Nicotiana benthamiana
(single leaf treatment)

NPs functionalized with FITC
(fluorescein-isothiocyanate)
diluted in 0.1% EIA
(Ethoxylated isodecyl alcohol)

Confocal microscopy analysis
Leica TCS SP8

Results



Empty NPs
FITC-NPs 1:20
FITC-NPs 1:5
FITC-NPs 1:1
FITC-NPs
Chlorophyll autofluorescence
Free FITC 1:1000
Supernatant FITC-NPs 1:20
3D
Max. intensity projection
Overlay with brightfield

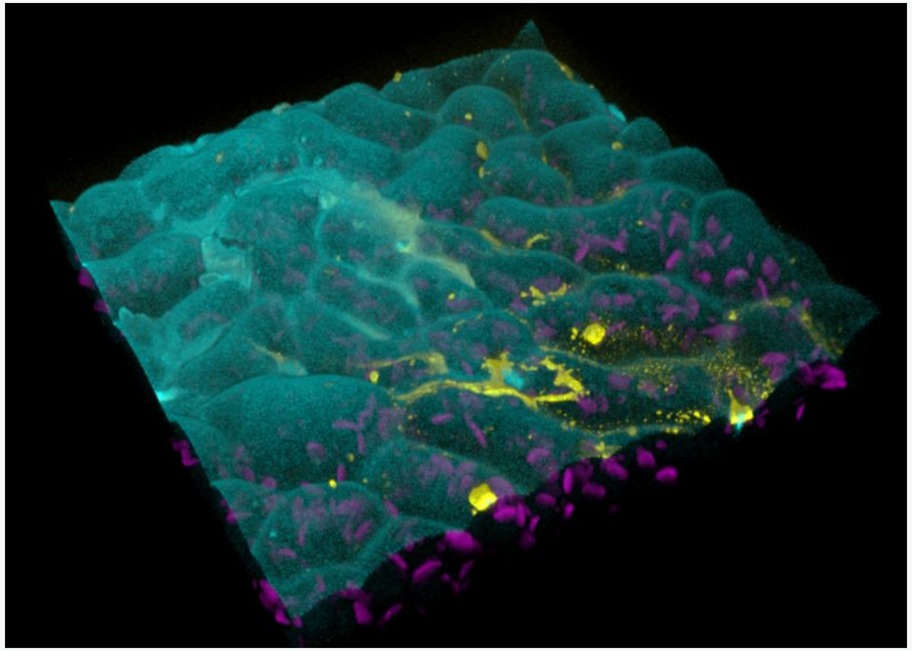
A. hybridus Preliminary results

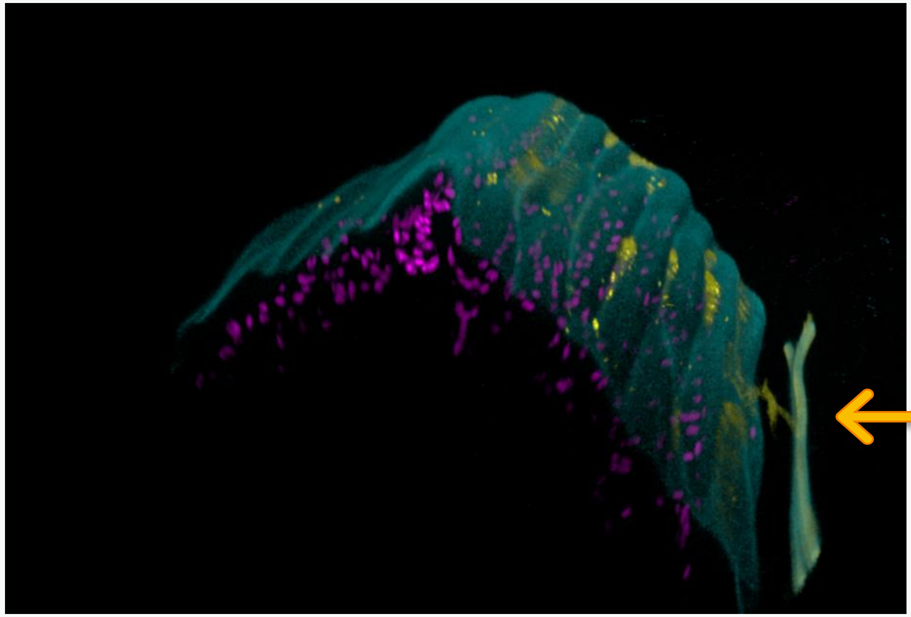
Adaxial surface

FITC-NPs

Chlorophyll autofluorescence

Cuticular lipid autofluorescence





Abaxial surface – detail of leaf vein

Deposition of NPs

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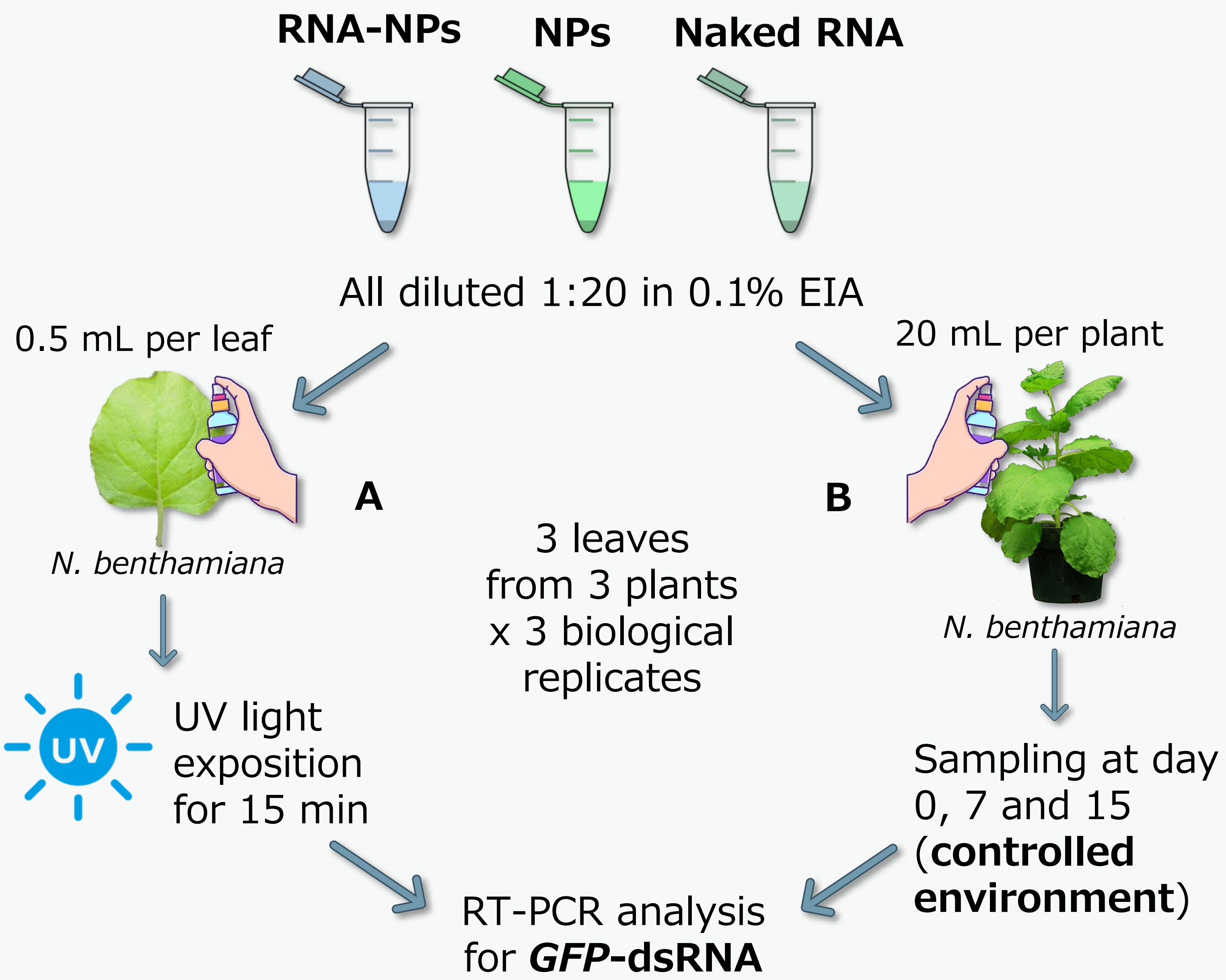
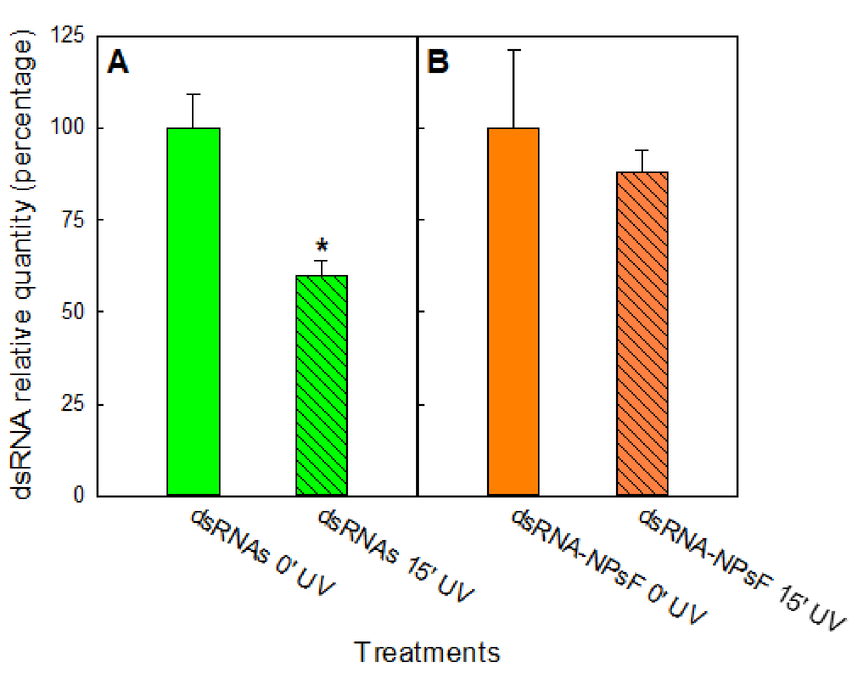
Methods

2.Assessment of the ability of NPs to protect dsRNAs from degradation



Results

A



NPs protect *GFP*-dsRNA from photo-degradation

B

Days after treatment	Treatment	GFP-dsRNA RQ
7	NPs	0.02 ± 0.005
7	Naked RNA	**0.93** ± 0.203
7	NPs-RNA	**0.96** ± 0.178
15	NPs	0.02 ± 0.004
15	Naked RNA	**0.79** ± 0.140
15	NPs-RNA	**0.94** ± 0.205

No statistical significance, but a trend is visible

Conclusions

1

The uniform distribution of NPs on leaf surface depends on:

- Leaf morphology
- NPs concentration, size and zeta potential

Do NPs enter the plant?

2

NPs are presumably able to protect dsRNA

Actually, it is necessary to study their performance in an open-air environment.

How are we going on?

Inhibition experiments on ***Botrytis cinerea*** using NPs functionalized with a specific dsRNA sequence (Nerva *et al.*, 2020) with interference activity

Study of the morphology of ***Amaranthus*** species to understand the interaction of NPs with leaf surface - future perspectives: **functionalized NPs for weed control**

Definition of a protocol for the purification and characterization of **exosomes**

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Thank you for your attention!