*Poster*

# A protocol for expressing plants' chloride transporters into yeast cells.


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## ABSTRACT

In the plant kingdom, chloride (Cl-) was defined as an essential micronutrient and, according to the results recently reported from our research Group, it has been also described as a beneficial macronutrient (1,2). Due to the similarity of their physicochemical properties Cl- and nitrate (NO3-) share membrane transport mechanisms and present strong interactions in plant cells. For example, a reduction of nitrate transport in Arabidopsis thaliana mutants gives rise to increased root chloride uptake. We propose that these phenomena respond to a compensatory mechanism aimed to regulate chloride homeostasis, possibly optimizing nitrogen use efficiency in plants under low NO3- availability (1,2). Presently, a single Cl- uptake transporter has been described in plants: AtNPF6.3 and the corresponding orthologous from other spp (3). In addition, the molecular mechanisms that regulate Cl- nutrition in plants remain unknown. Furthermore, functional characterization of plant Cl- transporters is a difficult task since: (i) it requires complex electrophysiological procedures in both plants and heterologous (e.g. Xenopus laevi oocyte) systems; (ii) commonly, plant knockout mutations exhibit unclear phenotypes.

In order to easily detect and quantify the activity of Cl- transporters and their regulatory partners, we will take advantage of the model microorganism Saccharomyces cerevisiae, which has very low Cl- transport ability in the 1.0 - 10 mM range (4). We intend to obtain yeast lines expressing recombinant probes sensitive to Cl- and to pH, respectively. As a proof-of-concept, genes encoding the A. thaliana AtNPF6.3 (NO3-selective) and the Medicago truncatula MtNPF6.5 (Cl-selective) transporters, will be expressed in these yeast lines under the control of an inducible promoter when cloned in the Gateway® pYES-DEST52 vector. Using the Gateway clonase II system these transporters as well as the recombinant Cl- and pH-sensitive fluorescent probes will be co-expressed on yeast cells.

Once the system is set up, fluorescence assays and microelectrode analysis will be carried out to characterize these and other candidate Cl- transporters. In the near future this protocol will allow also a fast screening of Cl- transporters after yeast transformation with a plant cDNA library and selection through fluorescence-activated cell sorting (FACS) using a flow cytometry.

## REFERENCES

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