Proton (H^+) pumps functioning: linking form and function

- Teresa Dias –
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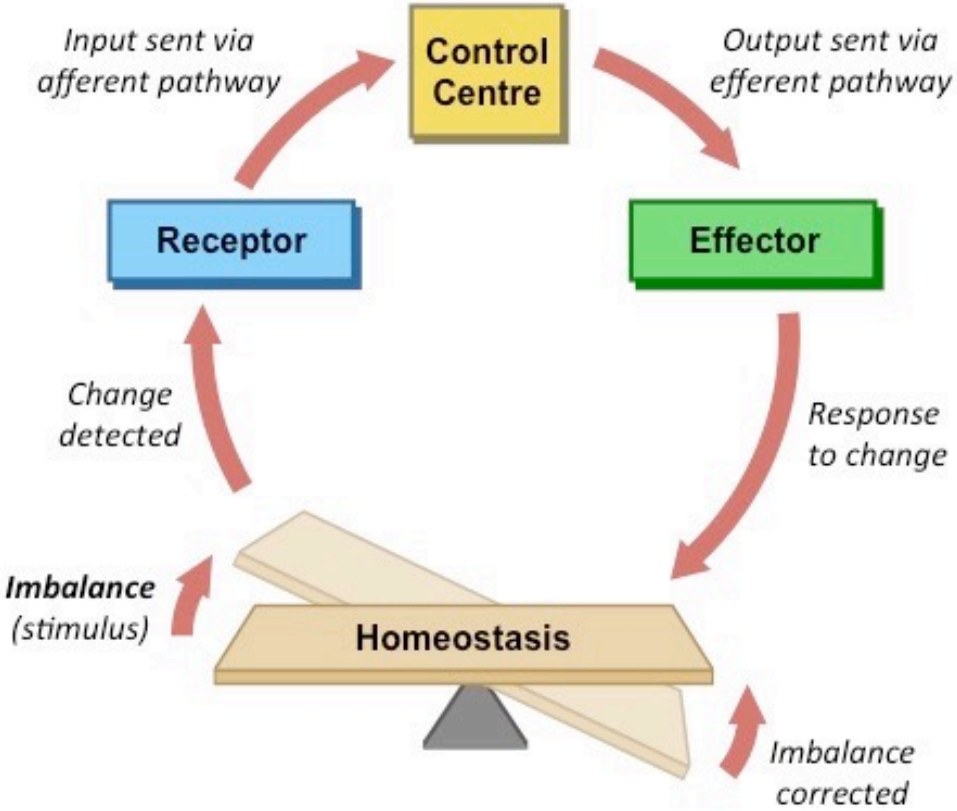
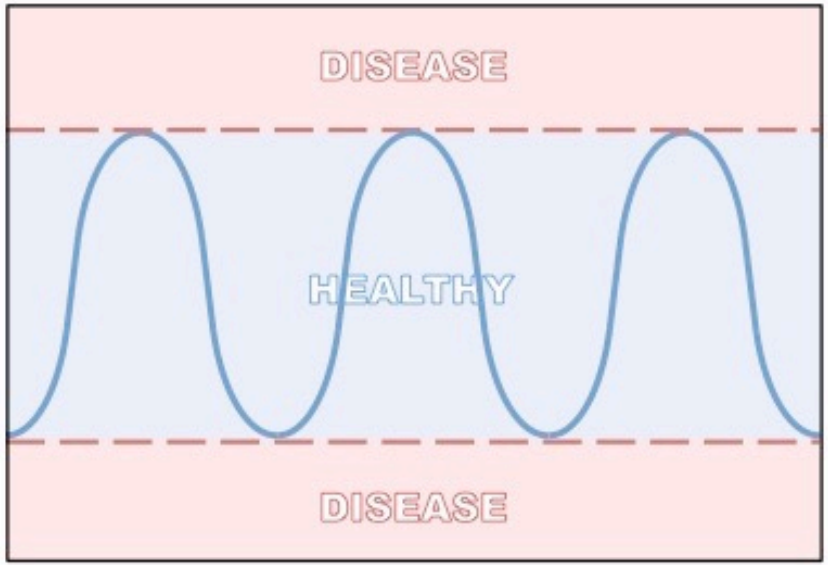




Homeostasis does **not** involve keeping conditions static
It involves keeping conditions within tightly regulated *physiological tolerance limits*

Negative feedback loops

Homeostasis

Homeostasis, from the Greek words for "same" and "steady," refers to any process that living things use to actively maintain fairly stable conditions necessary for survival.

Homeostasis has found useful applications in the social sciences.

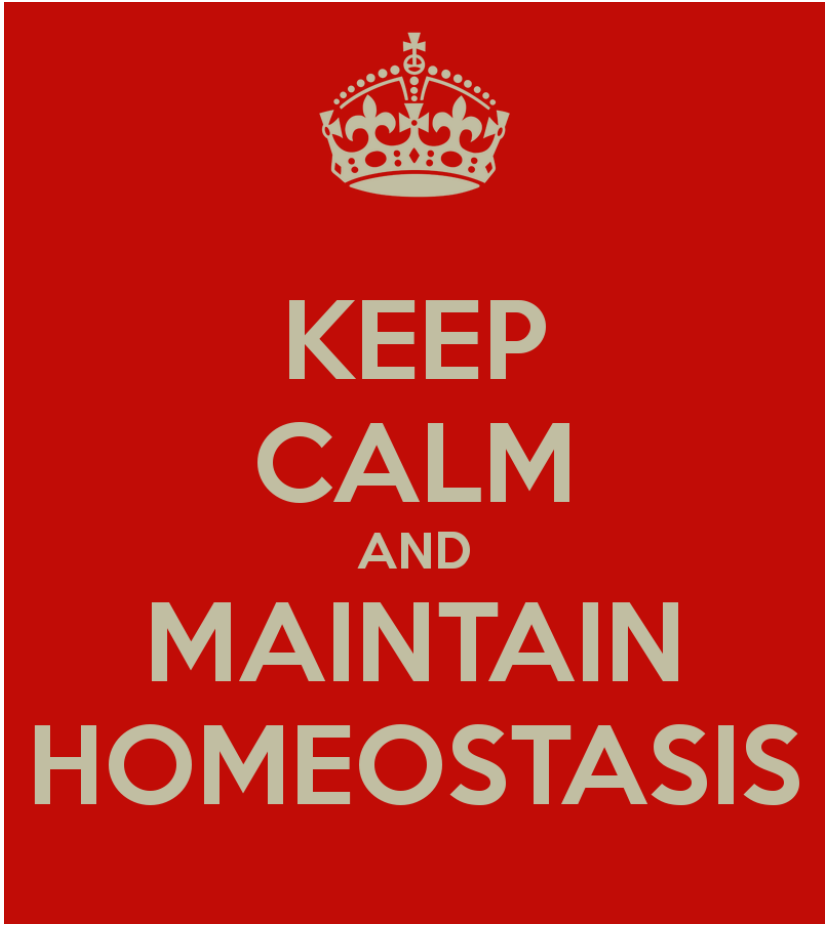
Examples:

Body temperature

Blood sugar

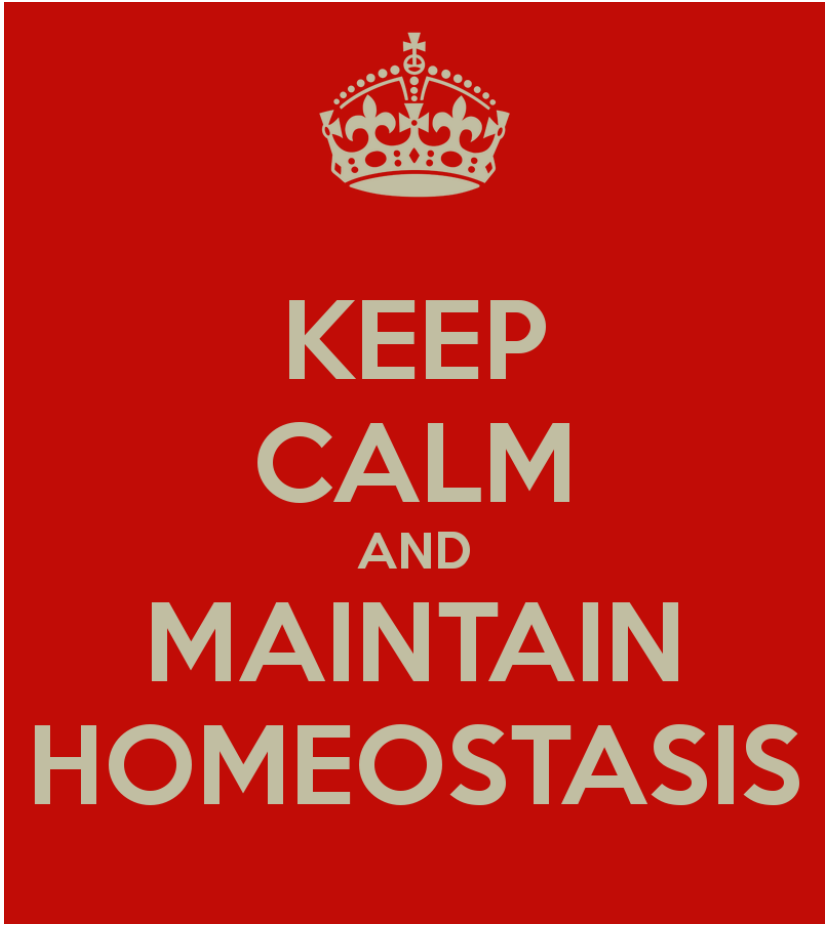
Cellular pH

Oscillation is a common and necessary behavior of many systems.



pH homeostasis

H^+ dictate the charge and structure of macromolecules and are used as energy currency by eukaryotic cells. The unique function of individual organelles therefore depends on the establishment and stringent maintenance of a distinct pH. This, in turn, requires a means to sense the prevailing pH and to respond to deviations from the norm with effective mechanisms to transport, produce or consume H^+ equivalents. A dynamic, finely tuned balance between H^+-extruding and H^+-importing processes underlies pH homeostasis not only in the cytosol, but in other cellular compartments as well (Casey et al 2010).



Cellular pH

Regulation and homeostasis of pH within a cell and its subcellular compartments are crucial for the viability of any living cell, from the simplest prokaryotes to complex multicellular organisms. Stringent pH requirements are necessary for efficient metabolism, protein stability, ion channel activity, membrane trafficking, protein sorting, and proteolytic processing of proteins. Cellular compartments are protected from rapid, local changes in pH by intrinsic buffering capacity that is provided by various intracellular weak acids and bases.

A more dynamic network for controlling intracellular pH is pH homeostasis, which is composed of an ensemble of well-coordinated ion carriers, such as ion permeable channels, transporters, and **H⁺PUMPS**.

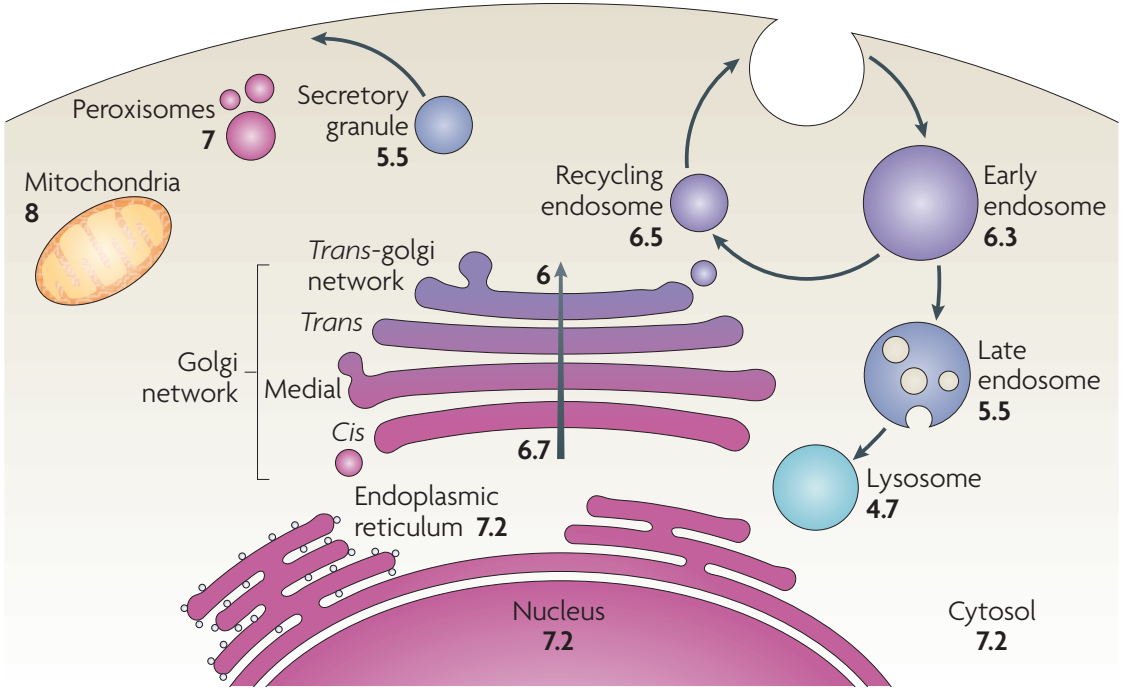


Figure 1 | **pH of the different subcellular compartments.** The pH of individual cellular organelles and compartments in a prototypical mammalian cell. The values were collected from various sources. The mitochondrial pH refers to the matrix, that is, the space contained by the inner mitochondrial membrane. Early endosomes refer to the sorting endosomal compartment. The pH of the multivesicular late endosome refers to the bulk luminal fluid; the pH of the fluid contained by the internal vesicles might differ.

Casey et al 2009

Quiz: How to study the pH of subcellular compartments?

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Review

Illumination of the Spatial Orde Genetically Encoded pH-Sensitiv

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PLoS one

In Vivo Determination of Organellar pH Using a Universal Wavelength-Based Confocal Microscopy Approach

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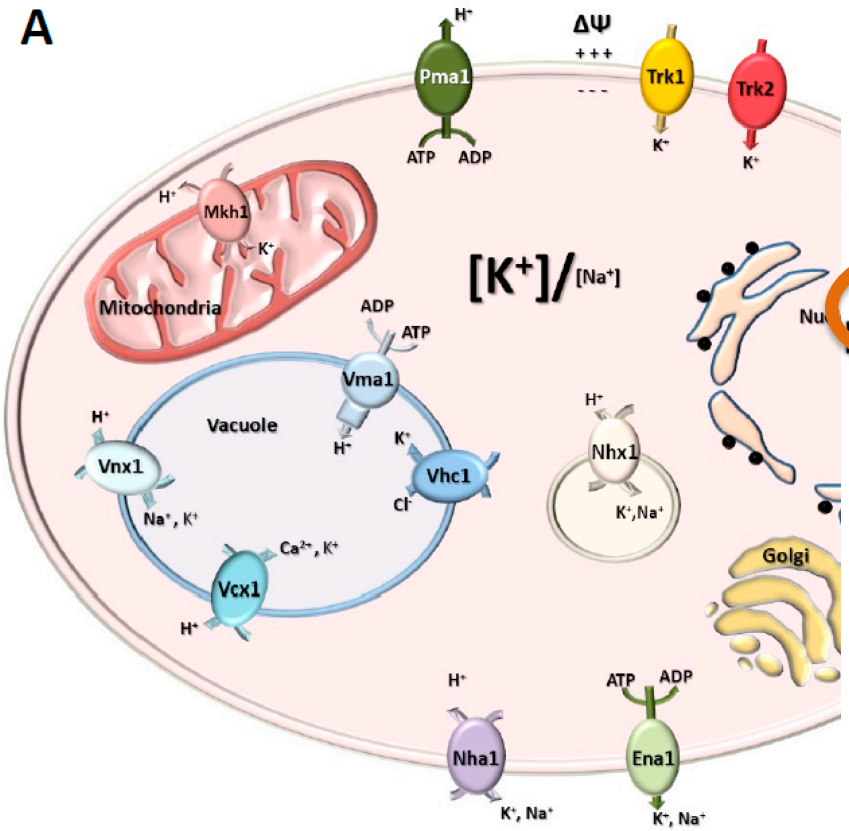
Abstract

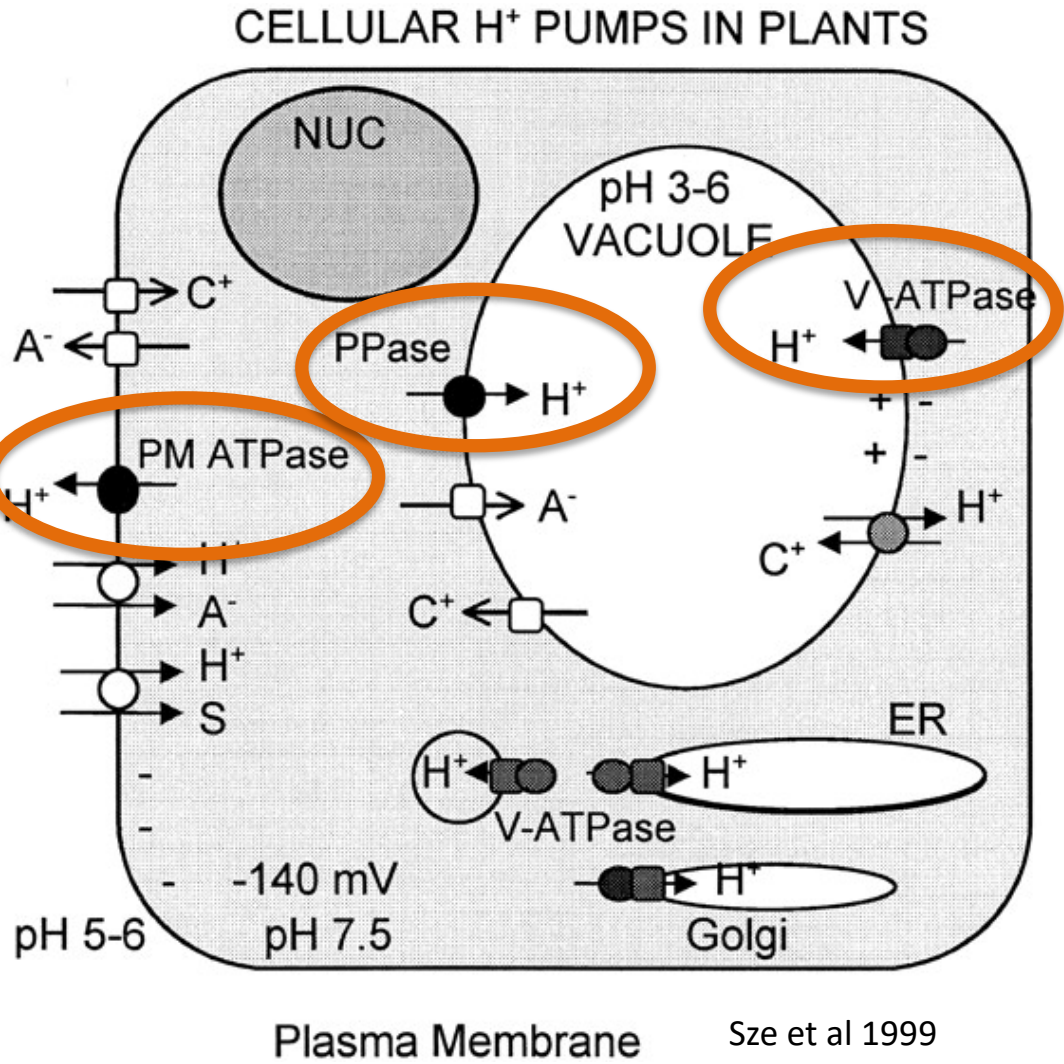
Many essential cellular processes are affected by transmembrane H^+ gradients and intracellular pH (pHi). The research of such metabolic events calls for a non-invasive method to monitor pHi within individual subcellular compartments. We present a novel confocal microscopy approach for the determination of organellar pHi in living cells expressing pH-dependent ratiometric fluorescent proteins. Unlike conventional intensity-based fluorometry, our method relies on emission wavelength scans at single-organelle resolution to produce wavelength-based pH estimates both accurate and robust to low-signal artifacts. Analyses of Ato1p-pHluorin and Ato1p-mCherry yeast cells revealed previously unreported wavelength shifts in pHluorin emission which, together with ratiometric mCherry, allowed for high-precision quantification of actual physiological pH values and evidenced dynamic pHi changes throughout the different stages of yeast colony development. Additionally, comparative pH quantification of Ato1p-pHluorin and Met17p-pHluorin cells implied the existence of a significant pHi gradient between peripheral and internal cytoplasm of cells from colonies occurring in the ammonia-producing alkali developmental phase. Results represent a step forward in the study of pHi regulation and subcellular metabolic functions beyond the scope of this study.


A S K
Always
Seek
Knowledge

Cellular H⁺ pumps and channels in yeast



Locascio et al 2019



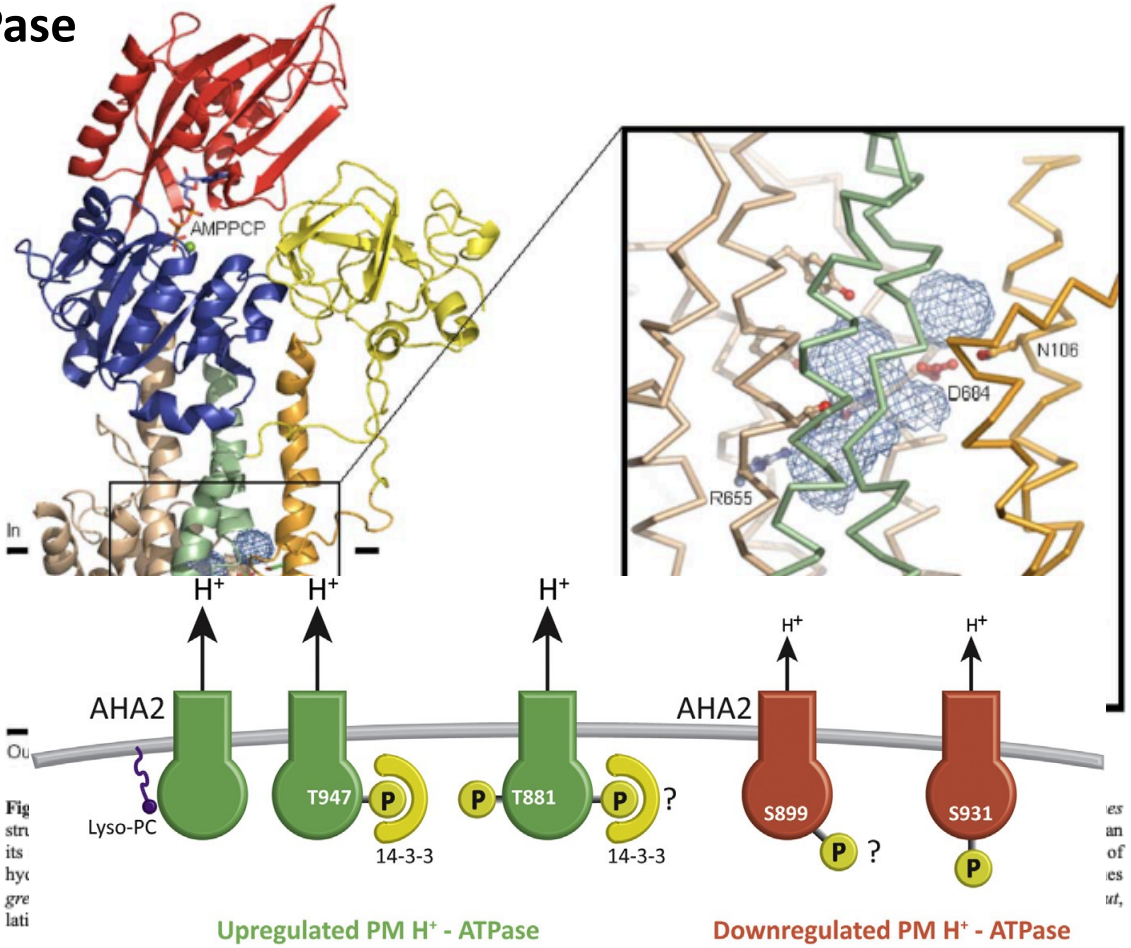
Sze et al 1999

Plasma membrane (PM) H⁺-ATPase

Plasma membrane proton pumps belong to a large superfamily of pumps termed P-type ATPases.

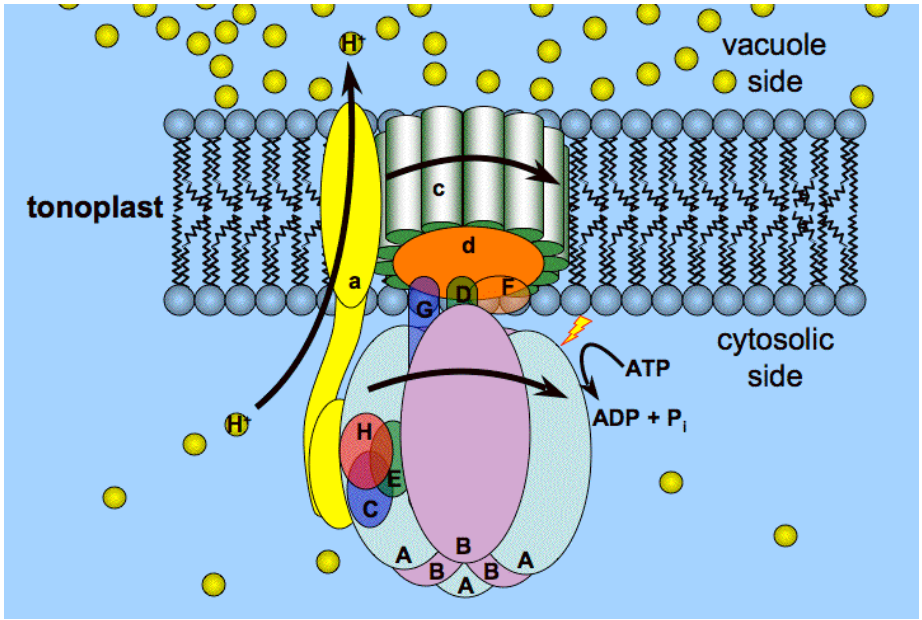
PM H⁺-ATPase is an electrogenic pump that exports cellular H⁺. In addition to generating a transmembrane chemical gradient of H⁺ (ΔpH; acidic on the outside), it also establishes an electrical gradient (the membrane potential; negative on the inside, up to the order of -300 mV in fungi), which is a prerequisite for growth.

Modification of the autoinhibitory terminal domains activates PM H⁺-ATPase activity, and on this basis it has been hypothesized that these regulatory termini are targets for physiological factors that activate or inhibit proton pumping (Falhof et al 2016).



Buch-Pedersen et al 2008

Vacuolar (V)-ATPase



V-ATPases are membrane-embedded protein complexes that function as ATP hydrolysis-driven H^+ pumps; are the primary source of organellar acidification in all eukaryotes, making them essential for many fundamental cellular processes.

Occur in: Vacuolar membranes in plants, yeast, other fungi; Endosomal and lysosomal membranes in animal cells; and plasma membrane of osteoclasts and some kidney tubule cells.

Mammalian V-ATPase subunits have multiple isoforms that are differentially localized to organelles, cells, or tissues. Mutations in isoforms are associated with autosomal recessive osteopetrosis, cutis laxa, and distal renal tubular acidosis (Vasanthakumar and Rubinstein 2020).

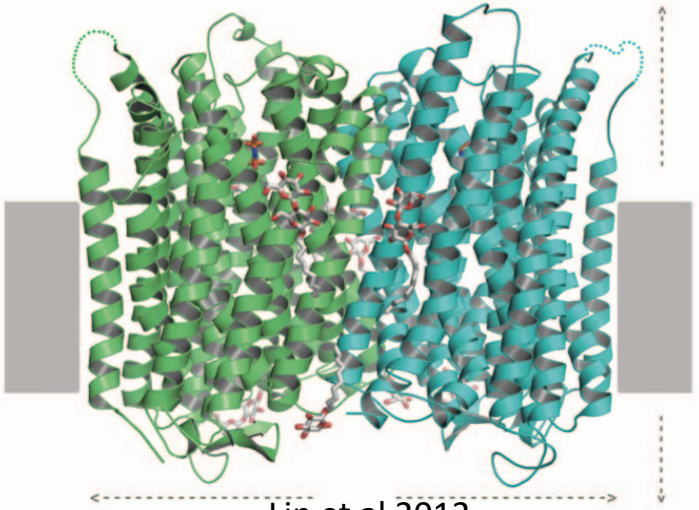
Until recently, proteins performing two disparate functions were considered an oddity and were regarded with suspicion. It is becoming increasingly clear that multi-tasking is a common feature of many proteins, and the V-ATPase is no exception. In addition to its canonical role as a generator of electrochemical H^+ gradients that can activate enzymes or drive substrate transport, we now also consider the V-ATPase as being a fusogen, as a transducer of information, and an anchorage site for the cytoskeleton and important metabolons (Maxson and Grinstein 2014).

Vacuolar Pyrophosphatase (H^+-PPase)

The H^+-PPase (pyrophosphatase) gene family is an important class of H^+ transporters that play key roles in plant development and stress resistance. The physiological and biochemical functions of H^+-PPases are ± well characterized.

H^+-PPases (proton-translocating pyrophosphatase) are a branch of membrane-bound pyrophosphatase enzymes that hydrolyze inorganic pyrophosphate (PPi) to obtain energy and transport protons across the membrane. These enzymes were considered to be unique to plants and photosynthetic bacteria, but recent studies have found that this gene family is also widespread among bacteria, archaea, and primitive and emerged in the Last Universal Common Ancestor (LUCA)

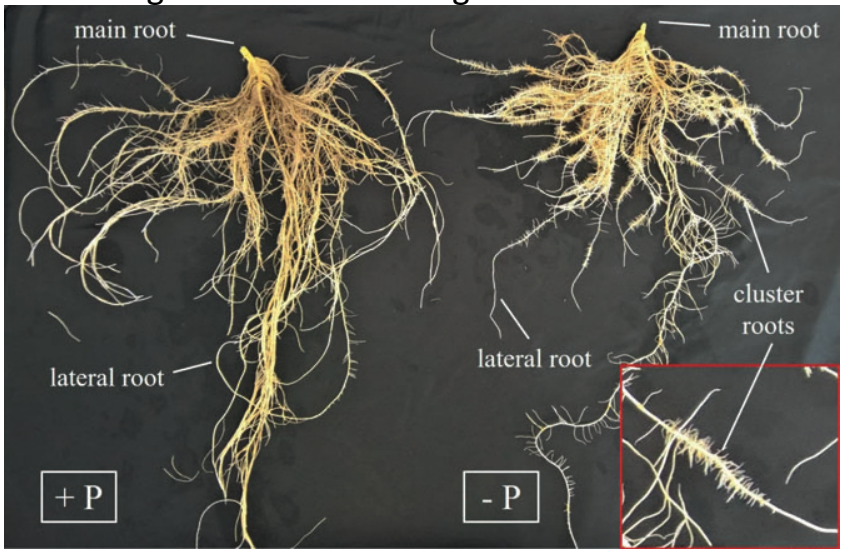
Most research on plant H^+-PPases has focused on the type I H^+-PPases, which are located on the vacuolar membrane and are thus known as vacuolar proton pyrophosphatases (V-PPase) Type I proteins are widely involved in metabolic processes such as the enrichment of metal ions in the vacuole [10] and hormone and nutrient transfer. Overexpression of type I H^+-PPase genes can significantly enhance the ability of plants to cope with abiotic stresses, such as anoxia or chilling, lack of nutrition, drought, and high salt level. This can also promote plant vegetative growth and produce plants with large biomass (Zhang et al 2020).



Lin et al 2012

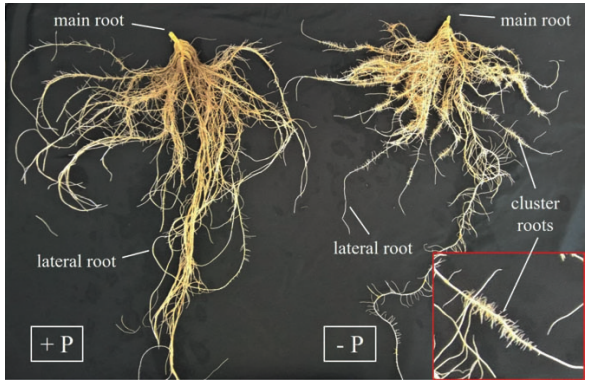
H^+-Pumps – examples linking form and function

To mobilize **PHOSPHATE** bound to soil particles, plants secrete organic acids such as **citrate**. In several plant species, upregulation of this process correlates tightly with increased PM H^+-ATPase activity, phosphorylation of Thr-947, and increased binding of 14-3-3 protein. Thus, by creating an outside positive membrane potential, PM H^+-ATPase appears to contribute to the charge balance during secretion of the organic acid.



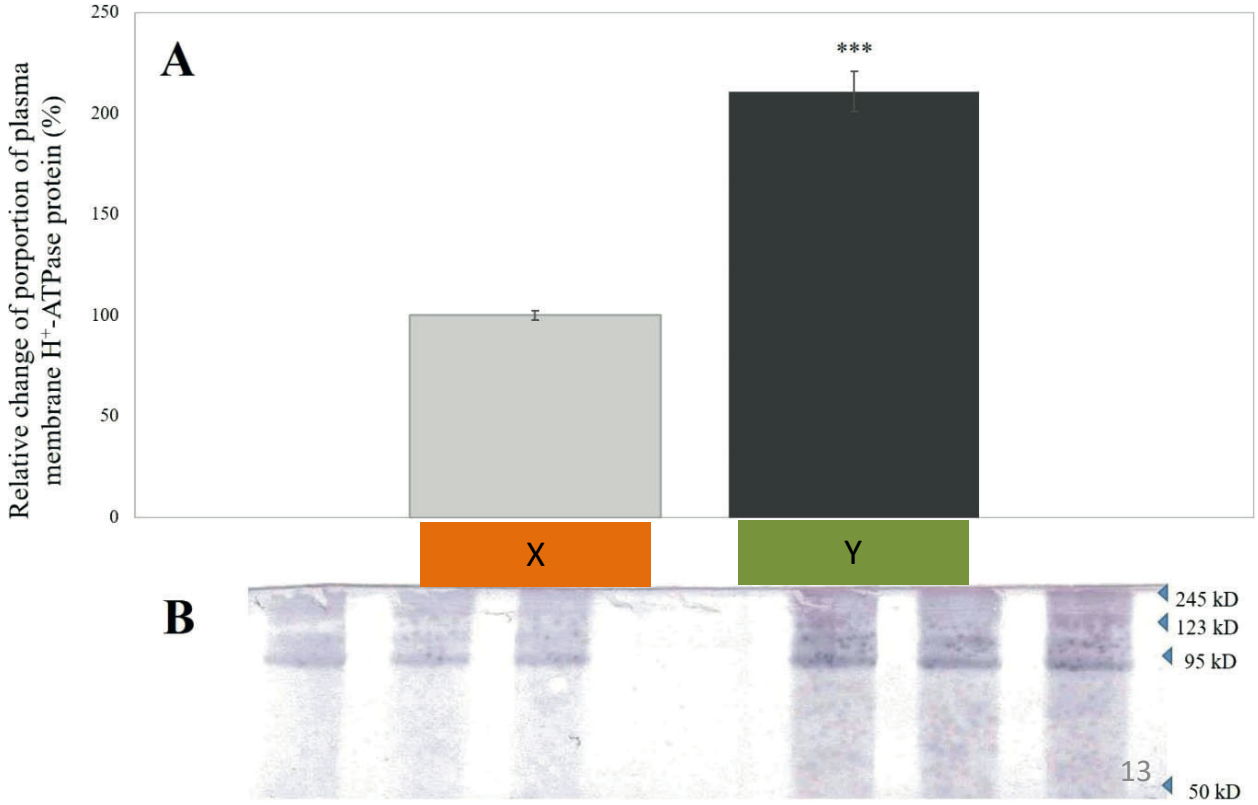
PM H^+-ATPases of active cluster roots are acclimated to phosphorus (P) deficiency. Cluster roots are second-order laterals with determinate growth, which are induced by P deficiency. Cluster roots enable the plant to acidify intensively micro compartments in the rhizosphere. H^+ are released by the PM H^+-ATPase, which helps to dissolve Ca phosphates and to provide low pH conditions necessary for activity of acid phosphatase (Stein et al 2019).

Quiz: identify the treatments

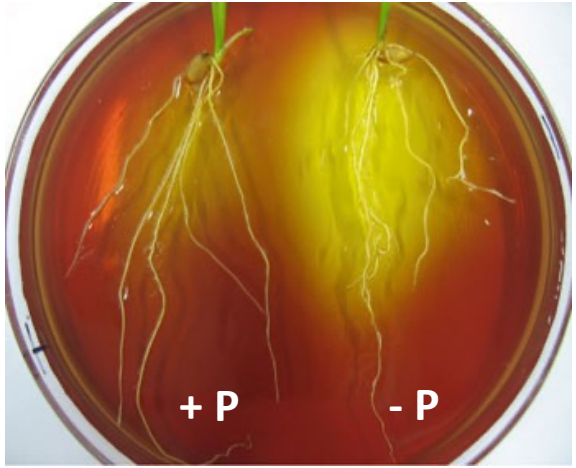


X – lateral roots (+P)
Y – cluster roots (-P)

Stein et al 2019



Quiz: identify the treatments



+ P

- P

Zhang et al 2011

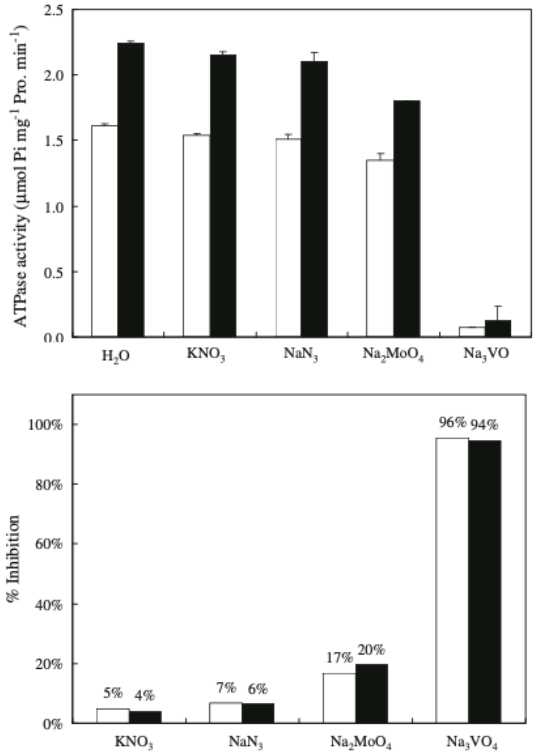


Fig. 2 Comparison of H^+-ATPase activity of plasma membranes derived from rice roots. Plants were supplied with and without P for 2 weeks. Plasma membrane H^+-ATPase activity was analyzed in the presence of 50 mM nitrate, 1 mM azide, 1 mM molybdate, and 0.1 mM vanadate at 30°C. Values represent means ± SE of three independent experiments

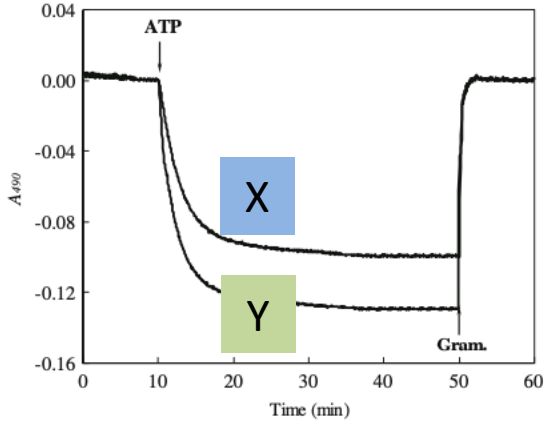


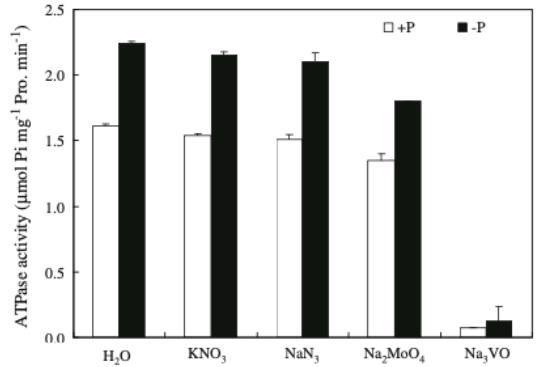
Fig. 4 Comparison of H^+ transport driven by plasma membrane H^+-ATPase across the plasma membranes. Membrane vesicles were isolated from P-fed and P-deficient rice roots. For the comparison of active H^+ transport, the pH gradient formation across vesicle membranes was monitored by A_{492} of AO. Intra-vesicular acidification was initiated by addition of 5 mM Mg-ATP. The established pH gradient was completely collapsed by 5 μM gramicidin (Gram.)

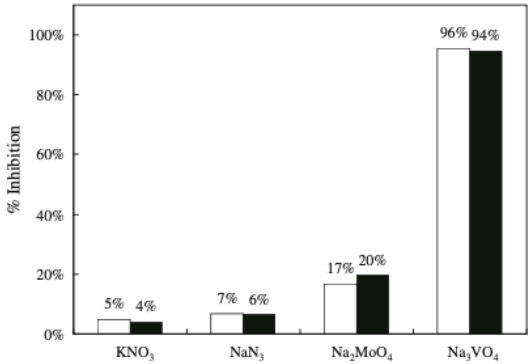
X: + P

Y: - P

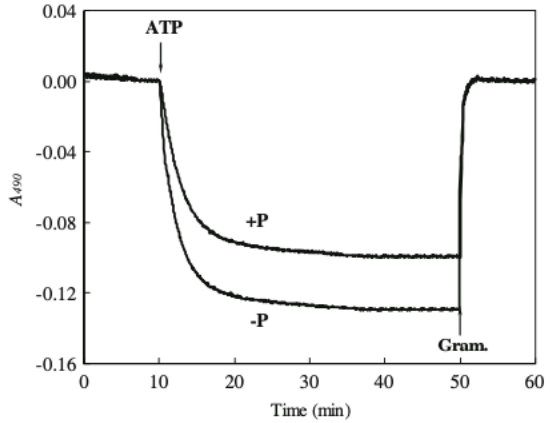
Quiz: How to measure H^+ pumps activity?

ATP/PPi hydrolisis





H^+ flux across membranes



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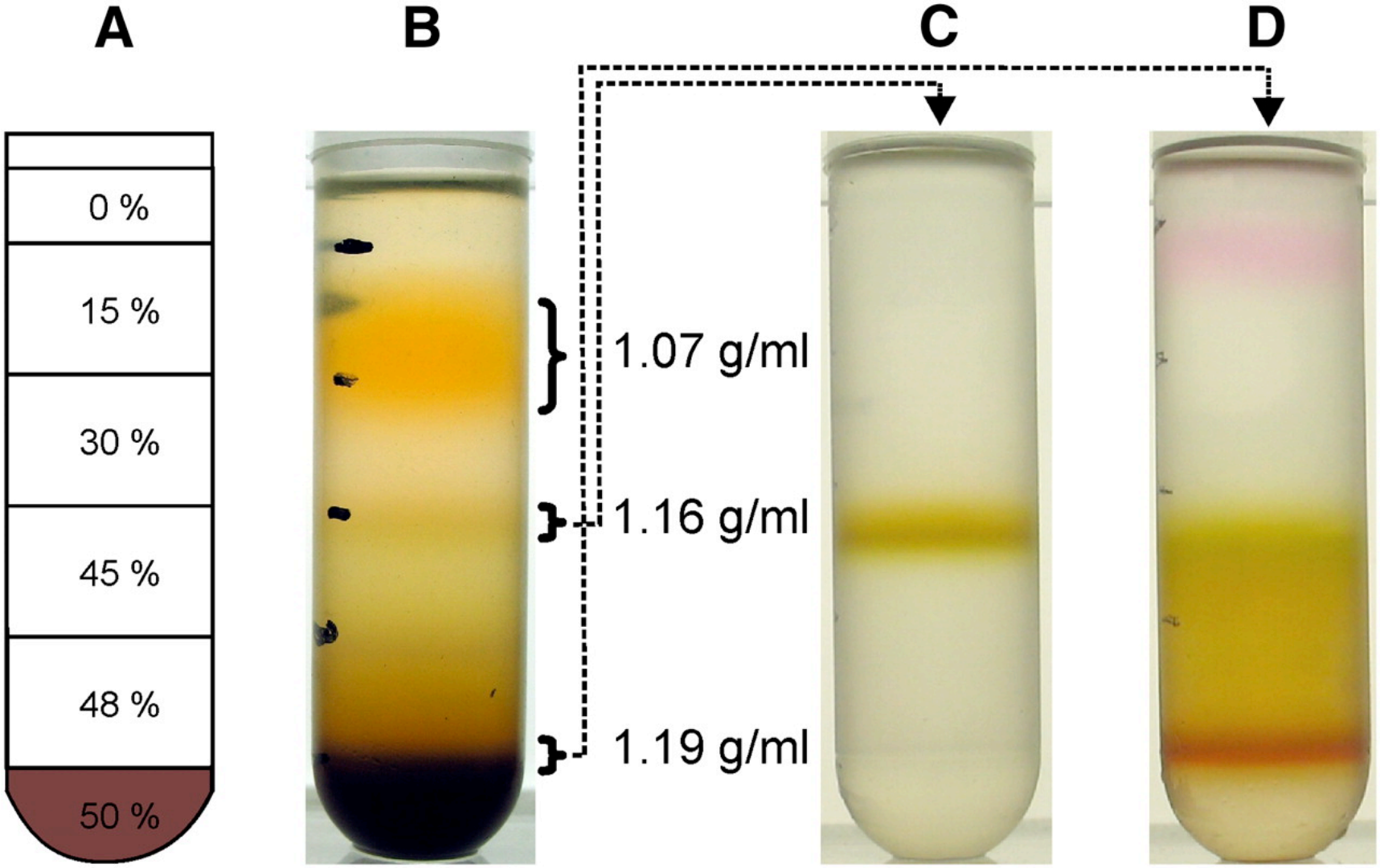

A
B
C
D
0 %
15 %
30 %
45 %
48 %
50 %
1.07 g/ml
1.16 g/ml
1.19 g/ml

Quiz: why use the different compounds in the extraction buffer?

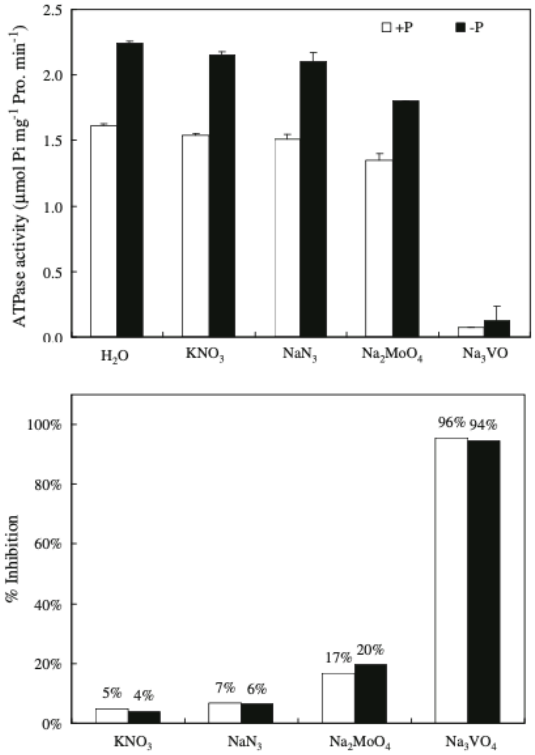
Briefly, about 15 g (fresh weight) of maize roots was homogenized using a mortar and pestle in 30 mL of ice-cold buffer containing:

- **250 mM sucrose**
- **10% (w/v) glycerol**
- **0.5% (w/v) PVP (PVP-40, 40 kDa)**
- **2 mM EDTA**
- **0.2% (w/v) BSA**
- **0.1 M Tris–HCl buffer, pH 7.6**
- **150 mM KCl**
- **2 mM DTT**
- **1 mM PMSF**

Quiz: why use the different compounds in the extraction buffer?

- **Sucrose** acts as a cushion, and gives better separation of cell fractionation, less contamination between these fraction;
- **Glycerol**, together with sucrose, has a cryoprotective effect on membranes;
- **PVP (polyvinylpyrrolidone)**, as polyphenol oxidase inhibitor;
- **EDTA (ethylenediaminetetraacetic acid)**, captures or "chelates" metal ions out of solution, preventing them from participating in unwanted side reactions;
- **BSA**, as a proteases substrate**;**
- **Tris–HCl buffer, pH 7.6**, to maintain pH homeostasis;
- **KCl**, has been commonly used in homogenization buffer and procedures of protein extraction because it facilitates the release of membrane-associated molecules**;**
- **DTT (dithiothreitol) and PMSF (phenylmethylsulfonyl fluoride)**, are powerful reducing agents.

Why measure enzyme activities in the presence of:



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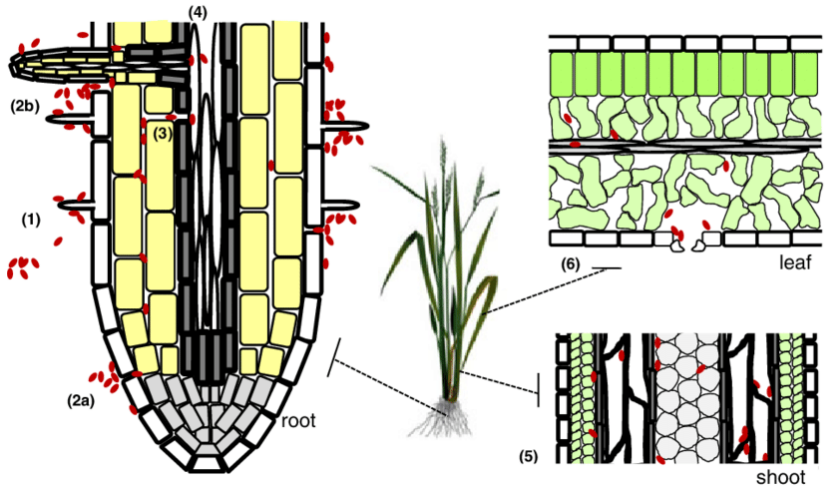
- H_2O: Control
- KNO_3: tonoplast V-ATPase inhibitor
- NaN_3: mitochondrial membrane H^+-ATPase inhibitor
- $NaMoO_4$: unspecific acid phosphatases inhibitor
- Na_3VO: PM H^+-APTase inhibitor

Linking form and function: plant inoculation with PGPB

Global change and growing human population are exhausting arable land and resources, including water and fertilizers.

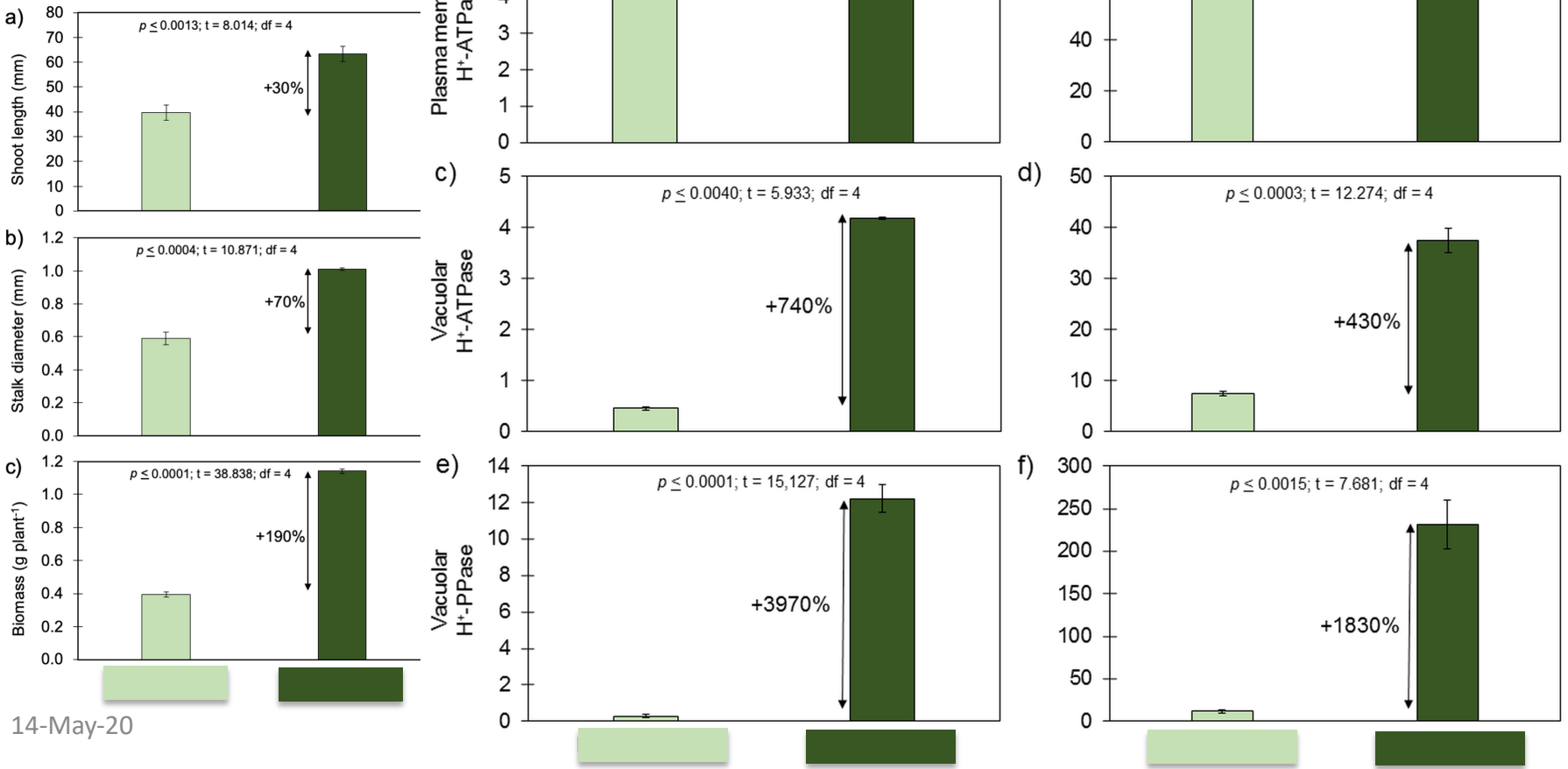
Here, we present a strategy for promoting growth, and nutrient and photosynthetic efficacy in rice (*Oryza sativa* L.): inoculation with the endophytic plant-growth promoting bacterium (PGPB) *Herbaspirillum seropedicae*.

Since plant nutrient acquisition is coordinated with photosynthesis and the carbon status of the plant, we hypothesize that **inoculation with *H. seropedicae* will stimulate proton (H^+) pumps**, and thus promote growth, nutrition and photosynthetic efficacy at low nutrient levels .



Ramos et al submitted

Linking form a



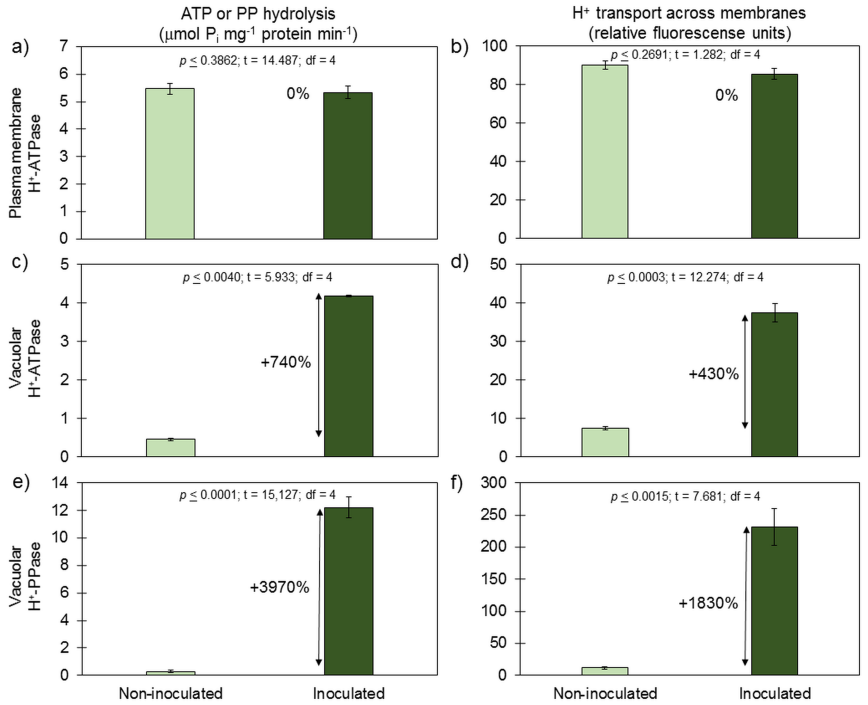
Linking form and function: plant inoculation with PGPB

The energy released during PPi hydrolysis (ΔG = 20–25 kJ mol^{-1}) is ~ 60% of the energy released by adenosine triphosphate (ATP) hydrolysis (ΔG = 35 kJ mol^{-1}), making PPi an alternative energy source when levels of ATP are low (Primo et al 2019).

The energetic backup system represented by the vacuolar H^+ pumps are tightly regulated in roots due to their higher energy demand for 'hosting' the endophytic PGPB and for lateral root proliferation.

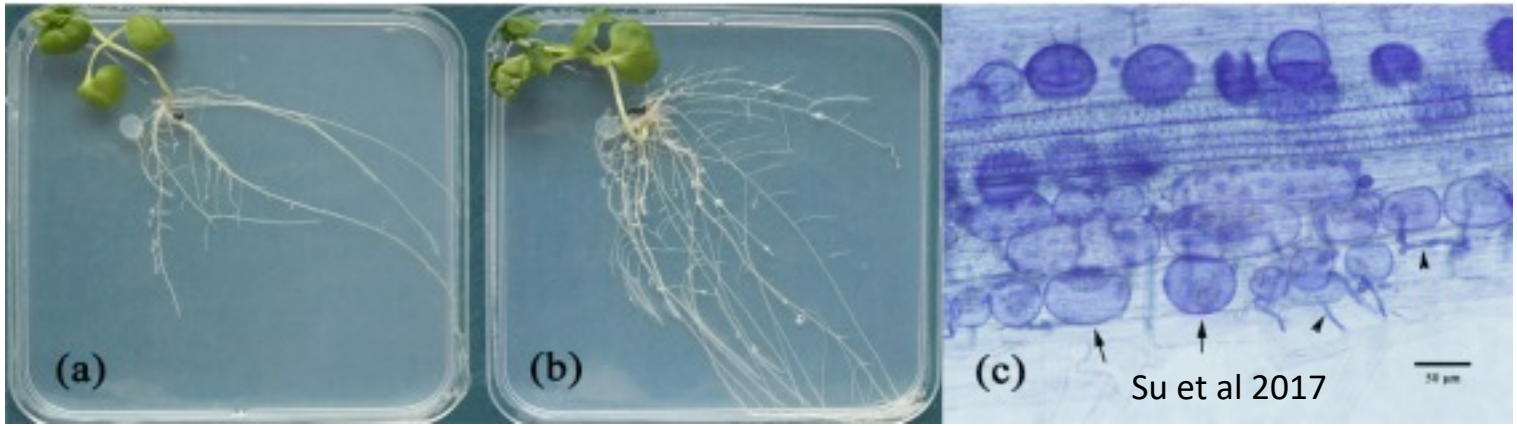
The activation of the PPi metabolism (H^+-PPase) in roots represents an energetic advantage, by saving the ATP needed to the cells with higher metabolic activity of physiologically strategic root regions (e.g. elongation zone and root hairs) .

Non-exclusively, higher H^+ pumping activity would enable greater vacuolar compartmentation of solutes and metabolites (including compounds which could inhibit cytoplasmic enzymes), which would increase vacuolar volume, allowing root cells to grow without *de novo* production of cytosolic components. Therefore, it is likely that *H. seropedicae* changed the abundance of vacuolar H^+ pumps by differential expression of genes and/or changed their activities due to post-translational modification or by interacting proteins (Ramos et al submitted).



Ramos et al submitted

Linking form and function: endophytic fungus free-living stage growth conditions

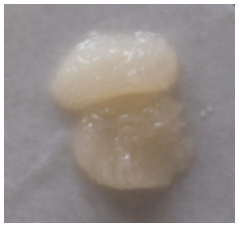


Serendipita indica (former *Piriformospora indica*) is a non-obligate endophytic fungus and generally a plant growth and defence promoter with high potential to be used in agriculture. However, *S. indica* may switch from biotrophy to necrotrophy losing its plant growth promoting traits.

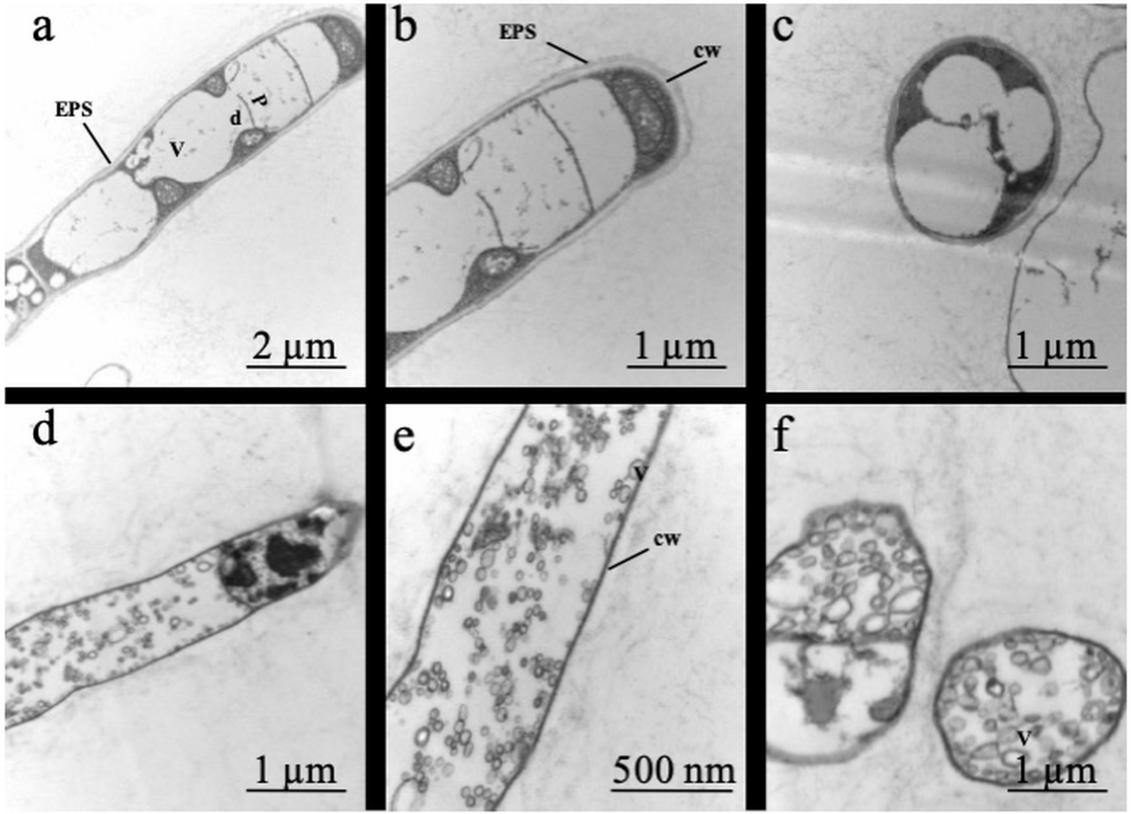
Our aim was to understand if the free-living stage growth conditions (namely C availability) regulate *S. indica*'s phenotype, and its potential as plant-growth-promoting-microbe (PGPM).

Dias et al submitted

Table 1 – Effect of two contrasting C availabilities (2 and 20 g L^{-1} of glucose or sucrose) on *S. indica*'s free-living stage phenotype. The two C sources originated similar phenotypes. Characteristics were studied in 10 colonies per replicate (n=3).

Characteristics	Compact phenotype 2 g L^{-1} (n=30)	Explorer phenotype 20 g L^{-1} (n=30)
Colony size	Small (< 1 cm)	Big (> 2 cm)
Colour	Pale Yellow	Pale Yellow
Shape	Spherical	Fringed
Surface type	Smooth	Rough
Texture	Compact	Fluffy
Appearance		

Dias et al submitted



Dias et al submitted

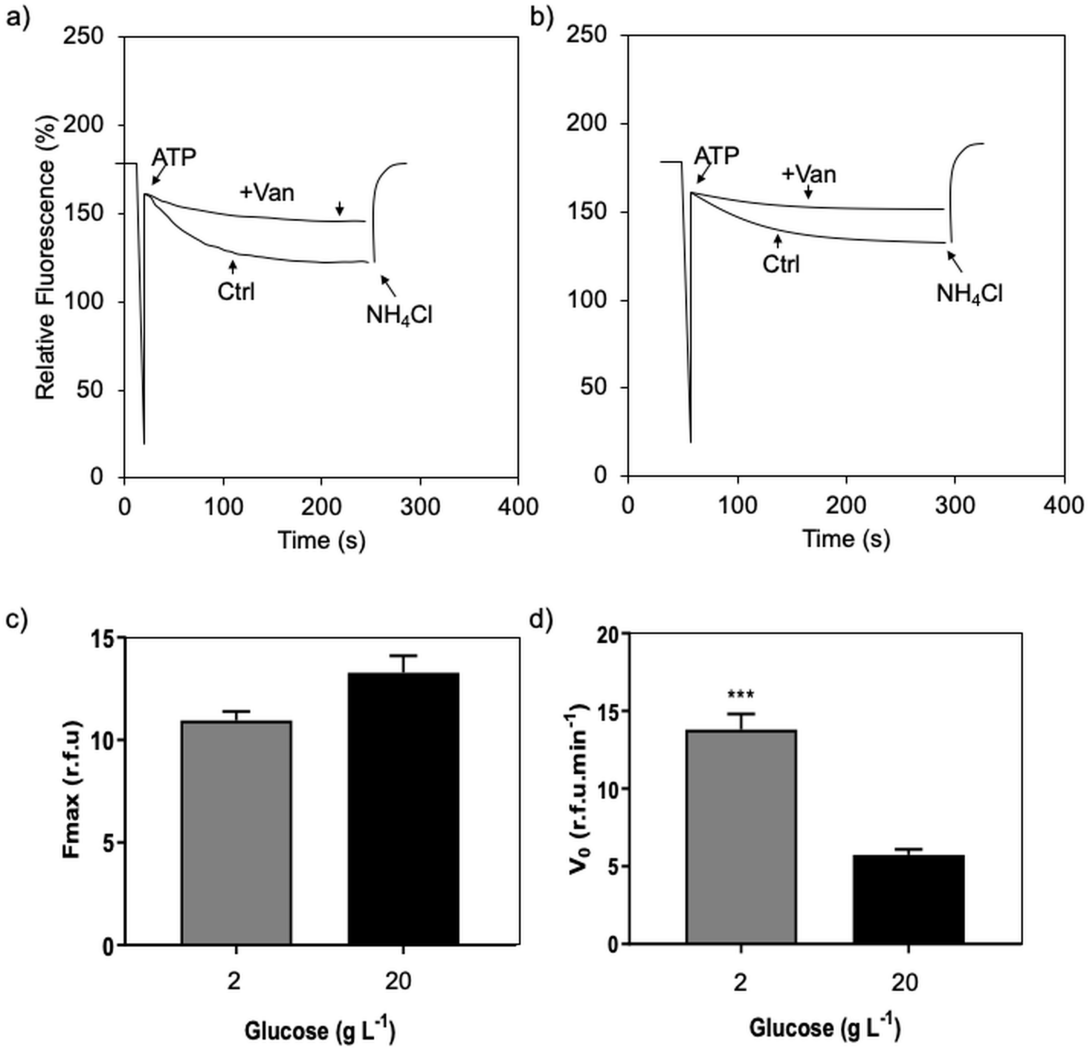


Figure 3 – Effect of two contrasting C availabilities (2 and 20 g L^{-1} of glucose) on *S. indica*'s (free-living stage) proton (H^+) transport of plasma membrane ATPase (P-H^+-ATPase): vanadate inhibition curve of the treatment of 2 g L^{-1} glucose (a); vanadate inhibition curve of the treatment of 20 g L^{-1} (b); maximum fluorescence (F_{max} – c) and initial velocity (V0) of P-H^+-ATPase H^+ transport (d). CTR-control, +Van- preincubation with 0.2 M vanadate. *** show significant difference by Student's t test ($p < 0.01$). Bars are the mean of 10 colonies per replicate (n=3) ± SD.

Dias et al submitted

Dias et al submitted

Low C availability (2 g L^{-1} of glucose) stimulated the potential activities of all the extracellular enzymes related with C cycling;

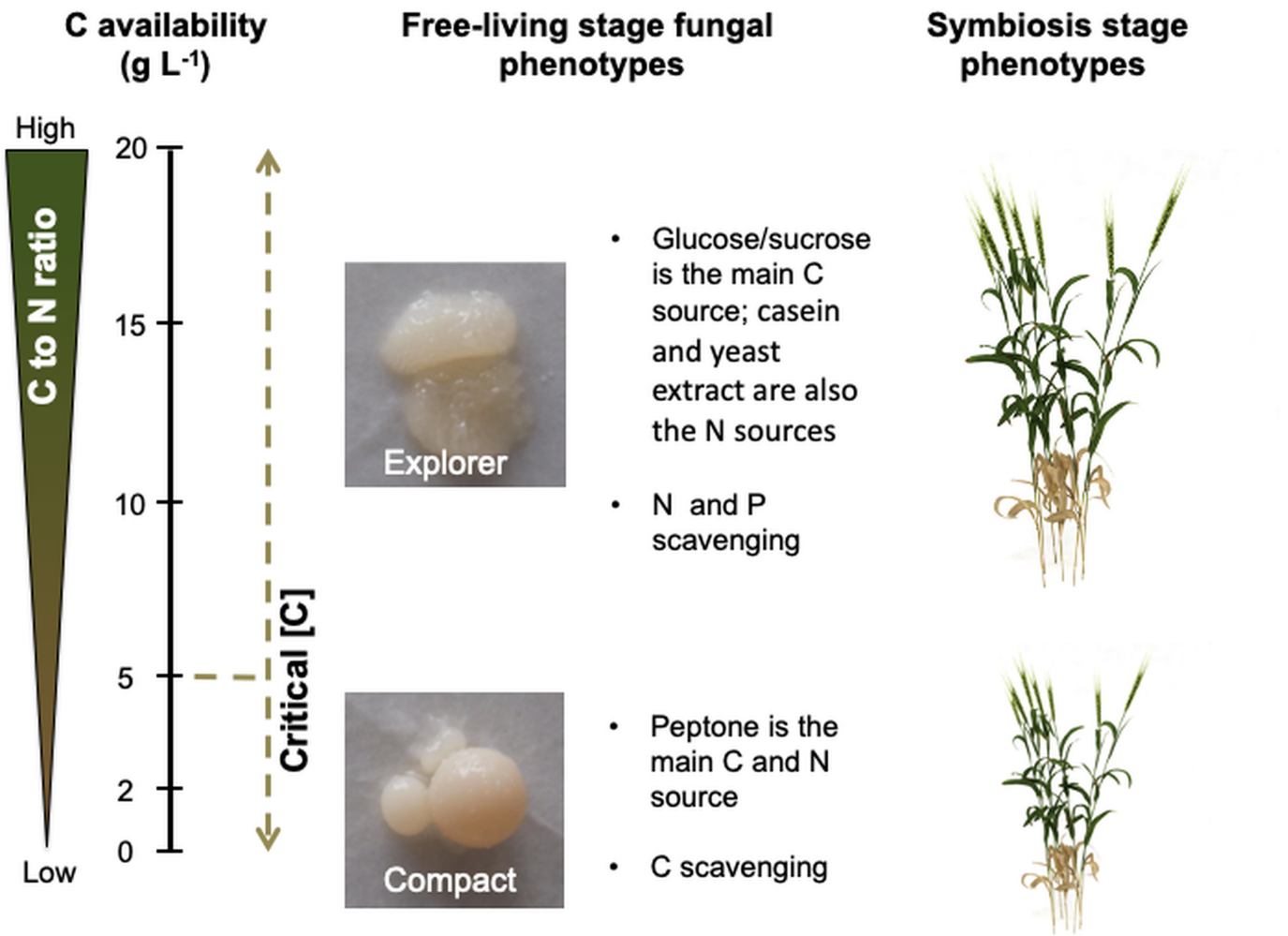
Higher C availabilities ($\geq$10 g L^{-1} of glucose) stimulated the potential activities of the extracellular enzymes related with N and P cycling.

The compact phenotype has a higher decomposing potential for C while the exploratory phenotype has a higher decomposing potential for N and P related substrates.

Table *2* – Effect of increasing C availabilities (glucose or sucrose) on the potential enzymatic activities of *S. indica*'s free-living stage. Potential enzymatic activities were expressed as µmol $h^{-1}g^{-1}$ mycelium. * shows significant effects among enzyme activities ($p < 0.01$). Different letters show significant differences between C availabilities ($p < 0.05$). Values are the mean of 10 determination*s* per replicate (n=3) ± SD.

[C] (g L^{-1})	2	5	10	15	20
			C cycling		
β -gluco *	110.6±13.1 a	48.7±5.9 b	62.0±12.2 b	42.5±5.9 b	44.2±2.2 b
β -xilo *	28.1±6.8 a	12.3±1.4 ab	8.4±1.4 b	5.5±2.4 b	5.0±2.2 b
β -glucu *	3.7±2.3 a	1.6±0.3 ab	2.2±1.0 ab	0.7±0.2 b	0.7±0.1 b
CeloBio *	36.8±2.3 a	16.0±2.9 b	16.7±1.5 b	16.1±1.7 b	15.8±2.8 b
			N cycling		
N- acetyl *	5.7±1.1 b	8.5±3.1 b	26.7±1.5 a	21.5±3.5 a	21.0±2.3 a
Leu *	7.6±1.8 b	7.8±2.1 b	28.8±1.4 a	25.8±1.0 a	25.5±0.0 a
			P cycling		
AP *	27.0±12.9 b	29.1±5.5 b	43.1±11.3 ab	57.2±2.7 a	52.9±2.9 a

β -gluco - β -glucosidase; β -xilo - β -xilosidade; β -glucu-β -glucuronidase; CeloBio- CeloBiohydrolase; N- acetyl - N- acetylgluosaminidase; Leu - Leucine aminopeptidase; and AP - Acid Phosphatase.



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Questions?