EFFICIENCY OF COMPLEX FORMATION BETWEEN ALUMINIUM AND MORIN OR LUMOGALLION IN THE PRESENCE OF ORGANIC LIGANDS

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Introduction

Morin and lumogallion were often used to stain and localise aluminium in plant tissues. But formation of the morin-Al complex is strongly influenced by the binding stage of Al and less is known about the Al-binding capabilities of lumogallion. Assessment of the constraints underlying Al-dye complex formation is especially important in case of Al-accumulating plant species as tea, where Al is supposed to be bound to organic acids.

- morin is more sensitive than lumogallion

* Results

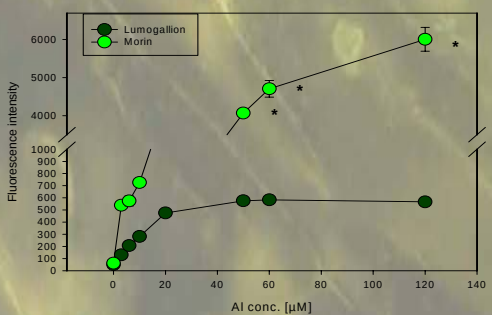

Fig. 1: Fluorescence intensity of lumogallion and morin at a concentration of 30 µM as affected by increasing Al concentrations. Lumogallion measurements at pH 5,2 in 0,1 M acetate buffer at excitation and emission wavelength of 507 nm and 567 nm. Morin measurements at pH 4,8 at excitation and emission wavelength of 418 nm and 502 nm; bars represent means +/- standard deviation of three replicates (* samples were diluted to avoid overshooting of the effective range)

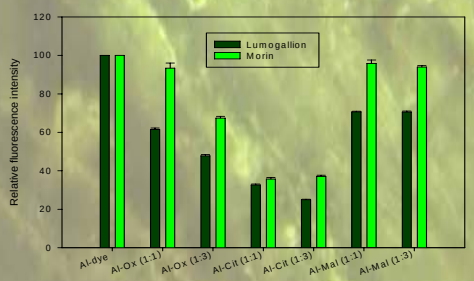

Fig. 2: Relative fluorescence intensity of lumogallion and morin as affected by the presence of different Al-chelating ligands in different ratios; Al-Morin: $AlCl_3$ 6 µM, Morin 30 µM; organic acids 6 or 18 µM, pH 4,8; detection at excitation wavelength 418 nm and emission 502 nm. Al-lumogallion: $AlCl_3$ 20 µM, lumogallion 60 µM; organic acids 20 or 60 µM, buffered by sodium acetate buffer pH 5,2; detection at excitation wavelength of 507 nm and emission 567 nm. Bars represent means +/- standard deviation of four replicates

Materials & Methods

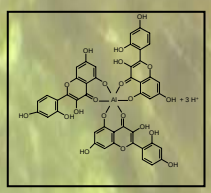

Fig.3: Al-morin complex

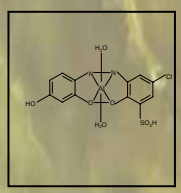

Fig.4: Al-lumogallion complex

***In-vitro experiments**:* For both dyes experiments were made to find the effective range for the complex formation by varying the dye concentration at one fixed Al concentration (data not shown). After combining Al and the organic ligands at ratios of 1:1 and 1:3 (Al/ligand) the samples were incubated at 25 °C for 0,5 h. Morin (33 mM), dissolved in DMSO was added to the samples to reach a concentration of 30 µM at pH 4,8. The Al-morin complex formation was performed at 25 °C for 1 h on a incubation shaker. Lumogallion (1 mM), dissolved in 0,1 M sodium acetate buffer pH 5,2 was added to reach a final concentration of 30 µM in the sample, after 1 h incubation at 60 °C on a incubation shaker fluorescence was measured with a Hitachi spectrofluorometer

Microscopy: Several month old seedlings of tea (*Camellia sinensis* var. sinensis) were treated in complete nutrient solution with 500 µM $AlCl_3$ at pH 4,2 for 24 h. 0,5 cm root tips were excised, rinsed with dd H_2O and embedded in frozen tissue tek and cut logitudinally by a kryo microtome at a chamber temperature of -20 °C and object temperature of -16 °C. Specimen (16 µm) were directly placed in a drop of the dye on a slide, and examined with a Zeiss Axioscope microscope.

- staining with both dyes shows symplastically accumulated Al-complexes

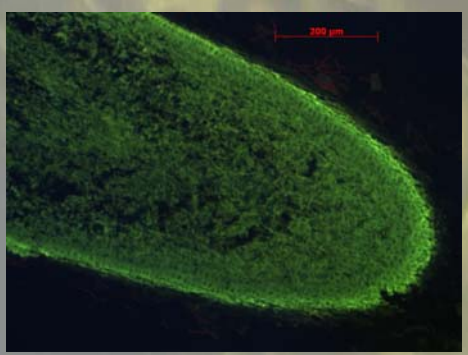

Fig. 5: Tea root tip treated with 500 µM Al for 24 h at pH 4,2, stained with 100 µM lumogallion, 162 ms exposure time with a bandpass of >450 nm and a longpass filter at 515 nm

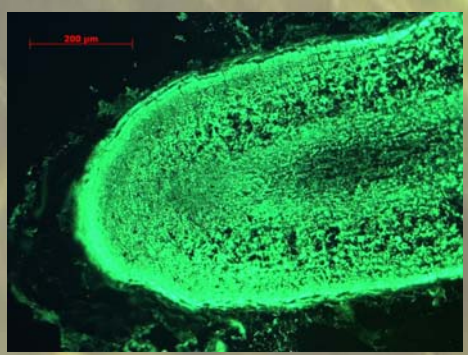

Fig. 6: Tea root tip treated with 500 µM Al for 24 h at pH 4,2, stained with 100 µM Morin, 162 ms exposure time with a bandpass of 395-440 nm and a longpass filter at 515 nm

- morin and lumogallion could not be used to differentiate between different binding forms of Al

Conclusions

Morin produces a stronger fluorescens intensity at same quantities of dye and Al than lumogallion. Lumogallion reaches its maxiumum fluorescence intensity at a dye to Al relation of 1:1, whereas morin has a still increasing intensity at relations greater than 1:1 suggesting that fluorescence intensity and complex formation are related to each other. Morin is able to produce one up to three fluorescence units per complexed molecule, depending on quantitative ratio. However, lumogallion produces only one unit per molecule.

The stability constant the of Al-morin complex is only slightly higher than that of the Al-lumogallion complex. For this reason it is not possible to distingunish between different Al-ligands using these two dyes. In plant species where oxalate is the dominating organic acid anion in the root tip as in case of tea and buckwheat both dyes could be useful tools for localizing Al. It is shown that morin is not as weak to sequester only monomeric inorganic Al as proposed by Lian et al. (2003)

Lian et al. (2003), Anal Bioanal Chem 376: 542-548