

Thesis title: *In vivo* detection of Ca²⁺ and pH in the apoplast and cytosol of plant leaves under salinity

Name: Sherif Hassan Morgan^{1,2}

¹Institute for Plant Nutrition and Soil Science, Christian Albrechts University, Kiel, Germany

²Plant Physiology, Plant Botany Department, Faculty of Agriculture, Cairo University, Egypt

ABSTRACT

Salinity is a global abiotic stress factor, which reduces crop growth and productivity. It is well known that an adequate level of Ca²⁺ can ameliorate salinity harmful effects. This study was conducted in order to better understand how calcium supply affects plant's behavior under salinity. Therefore, the ion relations were detected in leaves of wheat and faba bean. We especially focused on the effect of calcium supply under saline and non-saline conditions on leaf cytosolic calcium, [Ca²⁺]_{cyt}, apoplastic calcium, [Ca²⁺]_{apo}, cytosolic pH, pH_{cyt}, and apoplastic pH, pH_{apo}, with special reference to the hydrolytic H⁺-ATPase activity. This was done in order to study if there were any relations between the ion changes in the leaf apoplast and cytosol of plants grown under different conditions: control condition, C; salinity, S; calcium, Ca; calcium and salinity, S+Ca. Faba beans were additionally treated with potassium, K, or with potassium and salinity, S+K. Moreover, the ion dynamics were monitored in differently pre-treated faba bean plants directly upon salt addition to leaves or protoplasts.

Ratiometric methods were employed for detecting changes in the free ion concentrations of the apoplast and cytosol in plants grown under different conditions. Acetoxymethyl-esters of ion-specific dyes, Fura 2, SBFI, PBFI and BCECF, were used for [Ca²⁺]_{cyt}, [Na⁺]_{cyt}, [K⁺]_{cyt} and pH_{cyt}, respectively, while a dextranated Oregon Green was used for pH_{apo} detection. However, for [Ca²⁺]_{apo}, it was necessary to produce a Ca²⁺ pseudo-ratiometric probe (CG:LY) first by mixing dextranated Calcium Green, CG, with Lucifer Yellow, LY. By combining these two dyes, it is was possible for the first time to detect [Ca²⁺]_{apo} together with pH_{apo} after infiltrating CG:LY and OG separately.

In general, salinity alone decreased [Ca²⁺]_{cyt} in wheat, but not in faba bean. However, calcium supply under saline conditions increased apoplastic, cytosolic and overall Ca²⁺ in both wheat and faba bean and decreased the overall Na⁺ concentration. Moreover in wheat, calcium supply increased plant resistance to salinity, which was confirmed by the increase of the plants dry weight in both wheat cultivars differing in salt resistance.

In salt resistance wheat cv. Sedsl an alkalization were observed of pH_{cyt}, which is an indicator for salt resistance. Furthermore, cytosolic [Na⁺] remained low and was not altered by salinity. In contrast, [Na⁺]_{cyt} increased in less resistant wheat cv. Vinjett, but pH_{cyt} remained alkaline at the same time. The more obvious effect of calcium supply on improvement of salt resistance in wheat could be explained in terms of [Na⁺]_{cyt}/[Ca²⁺]_{cyt} ratio, which was reduced to a same level as compared to control plants.

In faba bean, usually salt stress leads to an apoplastic alkalization. But when calcium was supplied under saline growth conditions, apoplastic acidification combined with cytosolic alkalization, higher apoplastic and cytosolic Ca²⁺ were associated and lower cytosolic Na⁺, which finally resulted in higher hydrolytic H⁺-ATPase activity.

Therefore we conclude that these results can explain why calcium supply improves plants resistance to salinity stress.