Title
Mechanism of rhizosphere acidification in three plant species seedling

Authors
Cao, Jing
Zhang, Shen-qiang
Dong, Li-ping
et al.

Publication Date
2009-08-07

Peer reviewed

Introduction

Changes of pH in the rhizosphere are of major relevance from an ecological perspective as soil pH is a critical factor that influences the availability of many nutrients, the solubility of toxic elements and microbial populations in soil (Marschner, 1995; Hinsinger 1998). It is well known that legumes relying on symbiotic N_2 fixation acidify their rhizosphere to compensate for an excess of cations being taken up (Haynes, 1983; Marschner, 1995; Tang et al., 2003). In a previous study, however, Rao et al. (2000) found that some legume seedlings fed with nitrate acidified their rhizosphere even without fixing N_2. Acidification was observed only when shoots of plant seedling were exposed to light, whereas alkalization along the root axis occurred during the dark period. Further studies indicate that light-induced acidification in cowpea seedling is regulated by photosynthetic activity, but is not due to excess uptake of cations (Rao, 2002). In order to better understand this phenomenon, the specific nature of root exudation in relationship to photosynthetic activity was explored under normal conditions. We examined the contribution of other processes, such as root respiration and organic acid exudation, to rhizosphere acidification. In short, to investigate whether proton extrusion is specific to light condition in the three species seedlings, acidification of the rhizosphere induced by light in the presence of NO_3^- was compared with that induced by Al stress, respiration activities by root, photosynthetic activity and activity of plasma membrane H^+-ATPase.

Materials and methods

Three plant species seeds [Cowpea *(Vigna unguiculata* L. Walp. cv*),* soybean *(Glycine max* L.*),*and sunflower (*Helianthus annuus* L.)] were grown for one week in seed-pack growth pouches under controlled conditions in a growth chamber. During the growth period, one-quarter strength Hoagland nutrient solution containing 10 mM nitrate was supplied to all plants. A 3-mm thick agar gel (9·0 g l^{-1}) film containing bromocresol purple (0·1 g l^{-1}) was prepared. There were six treatments in the agar gel medium with bromocresol purple containing: (1) control with 1 mM potassium nitrate alone; (2) 100 •m/L $AlCl_3$ (To enhance the excretion of organic acids near the root apex); (3) 1 mM potassium nitrate and 100 •m/L $AlCl_3$; (4) 1 mM potassium nitrate and 10 mM SHAM (Inhibitors for respiration activities); (5) 1 mM potassium nitrate and 1 mM vanadate (Proton pump-inhibitors for photosynthetic activity); (6) 1 mM potassium nitrate and 5 mM DCMU solution sprayed on leaves during the incubation period (Inhibitor of photosynthetic activity to leaves). Each seedling was carefully taken from the growth pouch, and a single medium-sized root was selected. Subsequently, the single root was placed on a filter paper and pasted on the surface of the agar gel film in such a way that approximately three-quarters of the root surface was embedded in the gel. The initial pH of the gel was adjusted to 5.4-5.5 using 0.2 M NaOH. The agar gel films were covered with aluminum foil to avoid light access to the roots.

The agar gel films, together with the seedlings, were incubated for 6 hr in a growth chamber. Then, the agar gel films were scanned using a scanner with the settings at 90 d.p.i, full color and brightness level 3.

Results

Irrespective of treatments either with supplies of potassium nitrate alone or only $AlCl_3$ or both of them, localized occurrence of acidification was in the middle portion of the root axis in cowpea and soybean seedlings, and acidification occurred along the whole tap root in sunflower seedlings (Fig. 1). These results indicate that the acidification induced by light occurred in the three plant species and was probably due to relatively higher uptakes of cations in light conditions. The presence of Al alone caused a distinct rhizosphere acidification by all three plant species exposed to light. If this was derived from organic acid exudation was not studied here. Generally, at soil pH levels below 5.0 Al complexation with carboxylate released only in the root apex zones in response to elevated external Al concentration is a widespread mechanism for Al exclusion in many plant species (Matsumoto, 2000). The presence of Al had hardly any effect on the uptake balance of cations and anions; it seems that there is no interaction between potassium nitrate and Al toxicity.

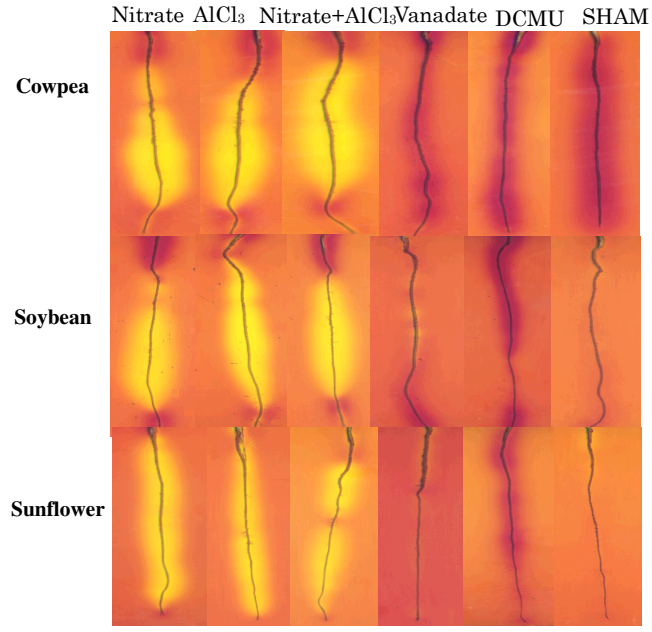

Fig. 1. Visualization maps of rhizosphere acidification or alkalization induced by plant root. Note: The colour gradients from yellow to purple indicate changes of rhizosphere pH from pH 4.0 to 7.0.

SHAM is an inhibitor of alternative pathway activity. Under shoots exposed to light rhizosphere acidification was fully inhibited by the application of SHAM in soybean and sunflower, but in cowpea the rhizosphere was alkalized weakly along the whole root axis. This shows that rhizosphere acidification may be related to the release of CO_2 from root respiration.

In comparison with the control treatment, the effect of proton pump inhibitors (vanadate) on the rhizosphere is a little bit dependent on species. The application of proton pump inhibitors stopped the light-induced acidification, and slight alkalization occurred along the tap root axis in cowpea seedlings. Light exposure of the shoot may increase the activity of the plasma membrane H^+-ATPase for proton extrusion. The inhibited intensity of light-induced acidification may be influenced by a concentration of proton pump-inhibitors and by differences in plant species. However, Rao et al. (2002) considered that the specific proton pump inhibitors of plasmalemma, DCCD and vanadate could not stop the light-induced acidification. This might be related to concentration of proton pump-inhibitors supply to plant species. The concentration of vanadate (1 mM) used in the present study was higher than that used by Rao et al. (200 μM vanadate, see Rao et al., 2002).

Light-induced acidification was completely inhibited when the photosynthesis inhibitor DCMU was applied to the leaves. Rhizosphere alkalization was observed along the whole root axis in all three plant species. In comparison, similar effects occurred in the three species when their shoots were exposed to darkness. This would suggest that cation uptake was decreased to a larger extent than anion uptake when the photosynthesis inhibitor DCMU was applied. Diurnal patterns of nutrient uptake have indeed shown a decreased uptake at night, with a steeper decrease for cations than anions (Le Bot and Kirkby, 1992; Macduff et al., 1997).

Conclusions

In three plant species exposed to Al^{3+} the phenomenon of rhizosphere acidification is similar as in a treatment with only potassium nitrate. The addition of Al^{3+} stress may not trigger the release of organic anions from plant roots. The release of protons is generally related to excess cation uptake; in the present study the induced acidification may be due to more uptake of cations in light. Organic acid exudation probably is not one of the factors which cause rhizosphere acidification in this study. On the other hand, according to our experiments it is concluded that the uptake of cations coupled with the process associated with root respiration could be one of factors causing the acidification in the studies plant species. Therefore, compared with the corresponding excretion of protons under the exposure of shoot to light, the contribution to rhizosphere acidification might be related to photosynthetic activity and proton pump of plasmalemma and CO_2 release from root respiration.

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