The release of biological nitrification inhibitors from sorghum root is regulated at the transcriptional level

Sorghum (*Sorghum bicolor*) roots release biological nitrification inhibitors (BNIs) to suppress soil-nitrifier activity and soil-nitrification. The presence of NH_4^+ in the rhizosphere stimulates the release of BNIs from roots and is hypothesized to be functionally linked to plasma membrane (PM) H^+-ATPase activity. However, whether the H^+-ATPase is regulated at the transcriptional level, and if so, which iso-forms of H^+-ATPases are involved in BNIs release are not known. Also, the stimulation of BNI release, whether it is due to NH_4^+ uptake or its assimilation in roots, is unclear, and it would be subsequently addressed by this study.

NH_4^+ concentrations up to 1.0 mM positively stimulated both PM H^+-ATPase activity and BNI release from sorghum roots; but at higher concentrations (>1.0 mM), NH_4^+ did not further increase BNI release and a decline in PM H^+-ATPase activity was observed (Fig. 1a, b). Vanadate, an inhibitor of H^+-ATPases, suppresses BNI release from intact sorghum plants (Fig. 1c). Twelve PM H^+-ATPase genes (iso-forms, designated as *SbA1 to SbA12*) were identified in sorghum genome; however, only five H^+-ATPase genes were stimulated by NH_4^+ in the rhizosphere. They have a similar expression pattern and is consistent with the observed variation in H^+-ATPase activity (Fig. 2). Methyl-ammonium (MeA), a non-metabolizable analogue of NH_4^+, had no significant effect on BNI release, H^+-ATPase activity, or the expression of H^+-ATPase genes (Fig. 3). These results suggest that the functional link between PM H^+-ATPase activity and BNI release is operational only at NH_4^+ concentrations of ≤1.0 mM in the rhizosphere. The variation in PM H^+-ATPase activity by NH_4^+ is due to transcriptional regulation of five iso-forms of H^+-ATPases. The stimulatory effect of NH_4^+ on BNI release is functionally associated with NH_4^+ assimilation and not from NH_4^+ uptake alone.

A mechanistic understanding of BNI release in sorghum helps in choosing suitable agro-ecological niche production systems where BNI function is expressed to its genetic potential for controlling soil nitrification. In addition, the use of slow-release N-fertilizers can allow soil ammonium levels ≤1.0 mM. This, coupled with the development of genetically modified crops with accelerated PM H^+-ATPase activity, can further improve BNI release from sorghum root systems to make production systems low-nitrifying and low-N_2O emitting with improved nitrogen-use efficiency, which in turn will be ultimately beneficial to human society and the environment.

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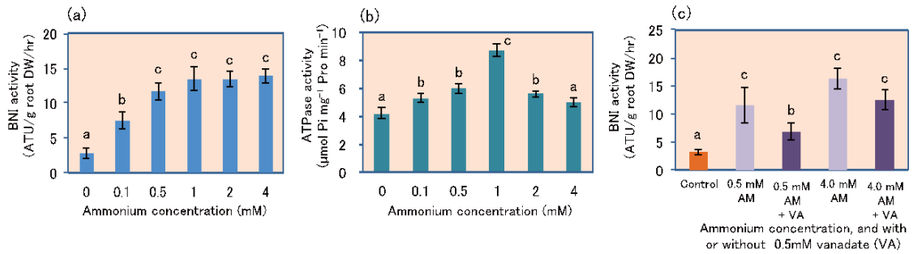

Fig. 1. The effects of ammonium (AM) on biological nitrification inhibitor (BNI) release from sorghum roots (a) and the plasma membrane (PM) H^+-ATPase activity in the roots (b), and the effect of vanadate (VA), an ATPase inhibitor, on BNI release from the roots (c)

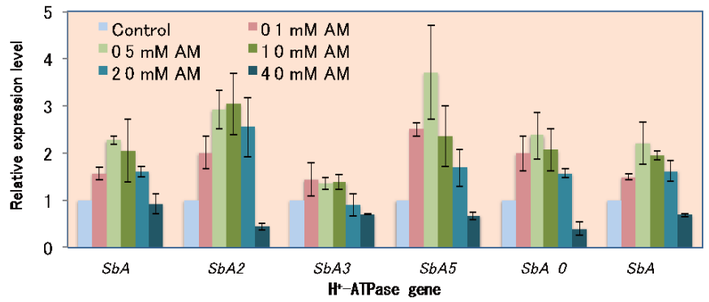

Fig. 2. The expression of six sorghum PM H^+-ATPase genes in response to NH_4^+ (AM) nutrition

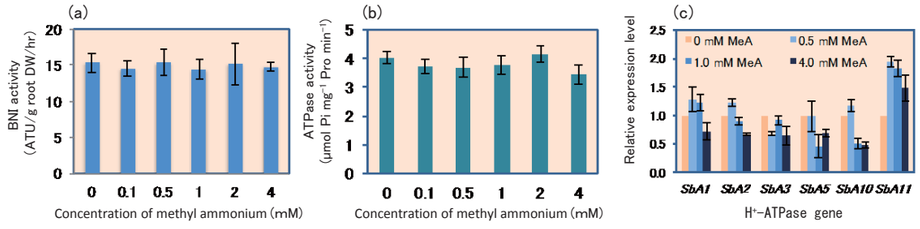

Fig. 3. The effect of methyl-ammonium (MeA), a non-metabolizable analogue to NH_4^+, on BNI release (a), the H^+-ATPase activity (b), and the expression of the H^+-ATPase genes in sorghum roots (c)