Molecular and physiological responses of recalcitrant indica rice to lignosulfonates during callus proliferation

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Abstract

Lignosulfonate (LS) is commonly used as stimulant to enhance plant growth. To date, the effects of LS on regeneration of recalcitrant *Oryza sativa indica* L. CV. MR219 has not been reported. Therefore, this study was undertaken to evaluate the effects of LS on callus proliferation of recalcitrant MR219 rice. The MR219 calli were proliferated on MS media supplemented with different ion-chelated (NaLS and CaLS) and concentrations (50, 100, 150, 200 mg/L) of LS. Optimum callus proliferation rate of 88% was successfully obtained on MS media supplemented with 100 mg/L CaLS. In addition, presence of CaLS also increased adventitious root formation of MR219 callus by 62% Further expression analysis of adventitious root-related genes (*OsWOX11*, *OsAUX1* and *OsIAA23*) recorded a 1.7-fold increment of *OsWOX11* expression in CaLS treated calli, implying a positive role of CaLS in adventitious root development. Besides, CaLS-treated calli also recorded a 1.2-fold higher endogenous indole-3-acetic acid (IAA) content and enhancement of nutrient ions (Na⁺, K⁺, Ca²⁺, Mg²⁺, Fe²⁺, Mn²⁺, Zn²⁺ and Cu²⁺) uptake as compared to non-treated calli. Consistently, expression analysis of auxin-related genes (*OsASA1*, *OsTAA1* and *OsYUC1*) and nutrient uptake-related genes (*OsAKT1*, *OsHAK5*, *OsCBL*, *OsCIPK23* and *OsCamk1*) also showed a similar increment trend. Interestingly, the Ca²⁺ increment was observed throughout four weeks, but the major increment of K⁺ was only detected starting from week two. The observed rise of Ca²⁺ following the enhancement of endogenous K⁺ content, further suggest the possible cross-talk between these ions. Subsequent, proteomic profiling analysis revealed, an increase of carbon and nitrogen metabolisms in CaLS treated callus. Taken together, our results suggest that the presence of CaLS enhance proliferation, and adventitious root formation of MR219 callus through up-regulation of endogenous auxin synthesis, enhance nutrients uptake, and carbon-nitrogenometabolisms.

Keywords: Adventitious root development; callus growth; carbon and nitrogen metabolism; lignosulfonates; nutrient uptake; *Oryza sativa*

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