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Full Length Research Paper

Increased heavy metal tolerance of cowpea plants by dual inoculation of an arbuscular mycorrhizal fungi and nitrogen-fixer *Rhizobium* bacterium

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Through biological inoculation technology, the bacterial-mycorrhizal-legume tripartite symbiosis in artificially heavy metal polluted soil was documented and the effects of dual inoculation with arbuscular mycorrhizal (AM) fungus and *Rhizobium* (N-fixing bacteria, NFB) on the host plant cowpea (*Vigna sinensis*) in pot cultures were investigated at six concentrations of Zn (0.0 – 1000 mg/kg dry soil) and Cd (0.0 – 100 mg/kg dry soil). From a number of physiological indices measured in this study, microsymbionts significantly increased dry weight, root : shoot ratios, leaf number and area, plant length, leaf pigments, total carbohydrates, N and P content of infected plants as compared with non infected controls at all levels of heavy metal concentrations. Tolerance index of cowpea plants was increased in the presence of microsymbionts than in their absence in polluted soil. Microsymbionts dependencies of cowpea plants tended to be increased at higher levels of Zn and Cd in polluted soil. Metals accumulated by microsymbionts-infected cowpea plant were mostly distributed in root tissues, suggesting that an exclusion strategy for metal tolerance widely exists in them. This study provides evidence for benefits of NFB to AM fungi in the protection of host plants against the detrimental effects of heavy metals. If so, bacterial-AM-legume tripartite symbiosis could be a new approach to increase the heavy metal tolerance of legumes plants under heavy metal polluted soil.

Key word: Mycorrhiza, *Vigna sinesis*, heavy metals, microsymbiosis, *Rhizobium*.

INTRODUCTION

With the development of industries, mining activities, application of waste water and sewage sludge on land, heavy metal pollution of soils is increasingly becoming a serious environmental problem. Phytoremediation (such as phytoextraction, phytostabilization and rhizofiltration) of soils contaminated by heavy metals has been widely accepted as a cost-effective and environmental-friendly cleanup technolology. However, the progress in this field is hindered by lack of understanding of complex interact-tions in the rhizosphere and plant –based mechanisms which allow metal translocation and accumulation in plant (Yu et al., 2004).

Complex interactions between roots, microorganisms and fauna in the rhizosphere have a fundamental effect on metal uptake and plant growth. The arbuscular mycorrhiza (AM) fungi are important rhizospheric micro-organisms. They can increase plant uptake of nutrients especially relatively immobile elements such as P, Zn and Cu (Ryan and Angus, 2003), and consequently, they increase root and shoot biomass and improve plant growth. It has been indicated that AM fungi can colonize plant roots in metal contaminated soil (Vogel-Mikus et al., 2005), while their effects on metal uptake by plants are conflicting. In slightly metal contaminated soil, most studies show that AM fungi increased shoot uptake of metals (Weinstein et al., 1995), while in severely contaminated soil, AM fungi could reduce shoot metal concentration and protect plants against harmful effects of metals (Malcova et al., 2003). Thus the benefits of mycorrhizae may be associated with metal tolerance, and also with metal plant nutrition. Therefore, in degraded and contaminated soils, that are often poor in nutrients and with low water holding capacities, mycorrhizae formation would be of great importance.

A biotechnological goal is to use a combined inoculation of selected rhizosphere microorganisms to minimize toxic effects of pollutants and to maximize plant growth and nutrition. Selected combinations of microbial