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SOYBEAN CO-INOCULATION WITH PLANT GROWTH-PROMOTING BACTERIA AS A BIOTECHNOLOGICAL TOOL TO REDUCE ARSENIC STRESS

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Arsenic (As) contamination in groundwater and agricultural soil is a significant global economic and health challenge. As has been reported to decrease root and shoot biomass, chlorophyll concentration, and inhibit nodulation and biological nitrogen fixation in soybean (*Glycine max* (L.) Merr.) in symbiosis with *Bradyrhizobium japonicum* E109. In the context of sustainable agriculture promoted by the Food and Agriculture Organization of the United Nations, co-inoculation of legumes with As-tolerant plant growth-promoting bacteria is proposed as a superior alternative to single inoculation. This study aimed to evaluate the effects of two bacterial strains previously selected for showing As tolerance and growth-promoting properties (*Pseudomonas* sp. AW4 and *Bacillus pumilus* SF5) on As accumulation and biometric parameters when co-inoculated with E109 in soybean. Bacterial strains were grown in tryptone yeast liquid medium until the exponential growth phase, adjusting their optical density (620 nm) to 1. Soybean seeds (BIOCERES 3.41 variety) were inoculated with bacterial suspensions at a dose equivalent to 7.5 mL kg⁻¹ of seeds and assigned to three groups: *B. japonicum* E109 (control), *B. japonicum* E109+ *Pseudomonas* sp. AW4, and *B. japonicum* E109+ *B. pumilus* SF5. Inoculated seeds were placed in trays containing filter paper with sterile distilled water and/or 12.5 µM arsenate (AsV)/arsenite (AsIII) equimolar solution, to assess the impact of co-inoculation on the early stages of soybean growth. Trays were incubated in a growth chamber at 28°C in the dark. After 8 days, germination percentage, germination index, germination speed index, root length, and relative root elongation were determined. Inoculated seeds were also sown in pots with a mixture of sterile perlite and soil (2:1) and watered with distilled water and/or 12.5 µM AsV/AsIII equimolar solution to assess the effect of co-inoculation at a more advanced phenological stage. Plants were grown in a growth chamber under controlled conditions (28±2°C, 16/8 h photoperiod, 231 µmol m⁻² s⁻¹ light, and 70-90% relative humidity). After 35 days (V4 phenological stage), chlorophyll content, root and shoot length, dry biomass, number of nodules and their

biomass, and As content in aerial tissues were measured. Both co-inoculations mitigated the effects of As on soybean from the early stages, promoting root elongation and germination rate. At a more advanced phenological stage, E109+ *Pseudomonas* sp. AW4 and *B. japonicum* E109+ *B. pumilus* SF5 co-inoculations alleviated As-induced stress by improving shoot length and chlorophyll content. Additionally, they significantly reduced As content in aerial parts. Results suggest that *Pseudomonas* sp. AW4 and *B. pumilus* SF5 are promising candidates for co-inoculation with E109 in As-contaminated soils, mitigating stress and contributing to safer food production.

Palabras clave: arsenate, arsenite, co-inoculation, soybean.