Effect of *Brevibacterium iodinum* RS16 and *Methylobacterium oryzae* CBMB20 Inoculation on Seed Germination and Early Growth of Maize and Sorghum-sudangrass hybrid Seedling under Different Salinity Levels

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Salinity is one of the most relevant abiotic factor limiting crop yield and its net primary productivity. In addition, salinity induces an increased stress ethylene synthesis in plants which, in turn, exacerbate the responses to the stressor. Bacterial single or co-inoculation effect was tested using previously characterized plant growth promoting (PGP) bacteria *Brevibacterium iodinum* RS16 and *Methylobacterium oryzae* CBMB20 on maize and sorghum-sudangrass hybrid under different concentrations of NaCl. Non-inoculated maize and sorghum-sudangrass hybrid showed 33.4% and 20.0% reduction in seed germination under highest NaCl (150 mM) level tested. However, under the same NaCl concentration, co-inoculation with *B. iodinum* RS16 and *M. oryzae* CBMB20 PGP strains increased the seed germination in maize (16.7%) and sorghum-sudangrass hybrid (4.4%). In Gnotobiotic growth pouch experiments conducted for maize and sorghum-sudangrass hybrid, co-inoculation of PGP *B. iodinum* RS16 and *M. oryzae* CBMB20 mitigated the salinity stress and promoted root length by 22.9% and 29.7%, respectively. Thus the results of this study could help in development of potential bioinoculants that may be suitable for crop production under saline conditions.

**Key words:** Salinity stress, NaCl, *Brevibacterium iodinum* RS16, *Methylobacterium oryzae* CBMB20, maize, Sorghum-sudangrass hybrid.

**Introduction**

Salinity is one of major abiotic stress in arid and semi-arid regions, more than half of the irrigated arable land area of the world are affected with salts, thus it substantially reduce crop yield by more than 50% (Bray et al. 2000). It is one of the most important factor limiting crop production, especially in sensitive crops (Zadeh and Naeini, 2007). Saline soils contain sufficient soluble salts to suppress plant growth through a series of interacting factors such as osmotic potential effect, ion toxicity and antagonism; they in turn induce nutrient imbalances (Neumann, 1995). Salinity stress affects all stages of crop growth, especially, seed germination and seedling growth stages are more sensitive for most plant species (Cuartero et al. 2006). Seedling growth of plant is the most critical stage in plant development and is highly responsive to the environmental conditions (Saritha et al. 2007). The effects of salinity on germination could be related to the imbalance in endogenous levels of growth hormones (Zholkevich and Pustovoytova, 1993; Jackson, 1997). Therefore, seeds with rapid germinating ability under salinity stress may be expected to achieve seedling establishment and more salinity tolerance and hence higher yields (Munns, 2002; Bybordi and Tabatabaei, 2009).

Plant growth promoting rhizobacteria (PGPR) are a group of bacteria that actively colonize the rhizosphere soil or in phyllosphere region and it helps in enhancement of plant growth and yield (Vessey, 2003). The mechanisms of plant growth promotion includes phytohormone production (Égamberdiyeva, 2007; Shaharooma et al. 2006), asymbiotic N₂ fixation (Salantur et al. 2006), production of siderophores, synthesis of antibiotics, enzymes and/or fungicidal compounds (Bharathi et al. 2004 and Jeun et al. 2004; Ahmad et al. 2007) and
solubilization of mineral nutrients (Cattelan et al. 1999). Under salinity stress, PGPR have positive effects on plants specifically on germination rate and seedling growth (Kokelis-Burelle et al. 2006). Importantly the PGPR containing 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase were considered to effectively facilitate the early growth of plants, especially under stressful conditions such as flooding, heavy metals, phytopathogens, drought and high salinity (Grichko et al. 2001; Belimov et al. 2005; Mayak et al. 2004). Ethylene is an important phytohormone connected with fruit ripening, however, overproduction of ethylene under stressful conditions can result in the inhibition of plant growth or death, especially for seedlings (Glick et al. 1997 and Ghosh et al. 2003). PGPR containing ACC deaminase can hydrolyze ACC, the immediate precursor of ethylene, to α-ketobutyrate and ammonia, leading to reduction in ethylene levels, thus promoting plant growth (Glick et al. 1998). In general, ethylene production, ACC concentration, ACC synthase (ACS), and ACC oxidase (ACO) enzyme activities increase with increasing levels of salinity stress. Inoculation with ACC deaminase producing PGPR reduced ethylene production, ACC concentration, ACS and ACO activities in salinity stress (Siddikee et al. 2011a). ACC deaminase- producing PGPR may assist plant growth by alleviating deleterious effects of stress ethylene. Moreover, PGPR are found to tolerate high salinity levels, which are often detrimental to growth of important crop plants (Bacilio et al. 2004).

The objective of this work was to examine in the effect of ACC deaminase producing PGP strains inoculation on seed germination and early growth of maize (Zea mays L.) and sorghum-sudangrass (Sorghum bicolor L.) hybrid under different salinity levels.

Materials and Methods

Bacterial strains and culture inoculum preparation

The halotolerant Brevibacterium iodinum RS16 was isolated from coastal saline soil of the Yellow sea, Incheon, South Korea. It possesses plant growth promoting (PGP) traits like nitrogen fixation and ACC deaminase activity (Siddikee et al. 2011b). The culture was maintained in Tryptic soy agar (TSA) medium supplemented with 5% (~0.85 M) NaCl (pH 7.2). For experimental studies 1.0 mL of the initially mass multiplied culture (1×10⁸ cfu mL⁻¹) was transferred to 100 mL fresh TSB supplemented with 5% (0.85 M) NaCl and allowed to grow for 24 h. The other bacteria used in this study was pink-pigmented facultative methylotroph (PPFM) Methylobacterium oryzae strain CBMB20, which was isolated from stem tissues of rice and well characterized in related to PGP activities (Madhaiyan et al. 2007). The culture was maintained in ammonium mineral salt (AMS) media with 0.5% sodium succinate supplemented at pH 7.2. For experimental studies, 1.0 mL initially mass multiplied culture (1×10⁸ cfu mL⁻¹) was transferred to 100 mL fresh AMS broth and allowed to grow for 72 h.

Effect of NaCl concentration on maize and sorghum-sudangrass hybrid seed germination

Maize seeds were surface sterilized by immersion in 70% ethanol for 1 min and 6% NaOCl for 5 min, followed by thorough rinsing with sterile distilled water (7-10 times). Sorghum-sudangrass hybrid seeds were surface sterilized by immersion in 70% ethanol for 2 min and 1% NaOCl for 3 min, followed by thorough rinsing in sterile distilled water (7-10 times). For conducting germination test, ten seeds were maintained per treatment on sterilized filter paper (Whatman No. 2) soaked in a 5 mL solution of 0, 50, 100 and 150 mM NaCl in Petri dishes. Treatment without NaCl addition was considered as control and each NaCl concentration was assigned as separate treatment, the experiment was conducted in triplicate. Germination potential was observed till 3 days at 28 ± 1°C under dark condition in a plant growth chamber (DS 54 GLP, DASOL Scientific Co., Ltd., Republic Korea). The number of germinated seeds was recorded every 24 h.

Bacterial inoculation effects on maize and sorghum-sudangrass hybrid seed germination under salinity stress

Germination potential of the seeds under different NaCl (50, 100 and 150 mM) concentrations was tested in presence of single inoculant or under co-inoculated condition. Surface sterilized maize and sorghum-sudangrass hybrid seeds were soaked in bacterial suspension (1×10⁸ cfu mL⁻¹) of each bacteria and imbibed for 4 h. Single inoculation treatments received 20 mL of culture as inoculum, and in case of co-inoculation, 10 mL of each culture were used. In case of control no seed bacterization was carried out. The rate of germination was estimated using a modified