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Original Research

Effect of Microbial inoculation in combating the aluminium toxicity effect on growth of *Zea mays*

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Abstract: The present study is aimed at improving the aluminium tolerance in maize crop employing the potential of microbial inoculants in conferring resistance to these toxicities via production of certain chelating compounds like siderophores, exopolysachharides and organic acids. Acid soils have now-a-days become one of the key factors for limiting growth of many agriculturally important crops. Aluminium is one of the major elements present in acid soils and is mainly responsible for toxicity in the soil. This aluminium is rapidly soluble in soil water and hence absorbed by plant roots under conditions where soil pH is below 5. This toxicity leads to severe root growth inhibition, thereby limiting the production of maize crops. It was observed that use of microbial inoculums can be helpful in elimination of these toxic compounds and prevent the inhibition of root growth . It was found that the soils contaminated with aluminium toxicity decreased the root length of maize plant significantly by 65% but *Bacillus* and *Burkholderia* inoculation increased this root length significantly by 1.4- folds and 2- folds respectively thereby combating the effect of aluminium toxicity. Aluminium concentration was found maximum in roots of plants which were grown under aluminium stress condition. But this aluminium accumulation decreased~2-folds when *Burkholderia* was used as seed inoculants under aluminium stress conditions. Also, at 60mM aluminium accumulation, phosphorus solubilisation in roots was found to be increased upto 30% on *Burkholderia* inoculation. However, *Bacillus* inoculation didn't show any significant difference in either of the case. Thus, the inoculation of seeds with *Burkholderia* isolates could prove to be a boon in sequestering aluminium toxicity in *Zea mays*.

Key words: Aluminium; Toxicity; *Burkholderia*; *Bacillus*; *Zea mays*.

Introduction

Zea mays (Maize), a large grain plant is predominantly a kharif crop but cultivated throughout the year in India due to its adaptability to wide range of climates. In India, maize stands as the third most important cereal crop after rice and wheat. In the Global Scenario also maize is the considered as the most important cereal crop as it contributes to food security in most of the developing countries. Around the world it grows from 48°N to about 40°S latitude. A well drained, deep, fertile soil having large water holding capacity is considered ideal for maize but if well managed it gives high yield on wide variety of soils. Soils having a pH range between 5.5 and 8.0 are preferred by maize, optimum range being 5.5 to 7.0 but below pH 5.5 solubilisation of aluminium ions occur. Many agriculturally important plant species have toxic effect of this solubilisation on their growth (1). Under such conditions, root growth and functions are primarily affected (2). Root length is reduced (3-7) which is presented through damaged lateral roots and root tips.

The roots damaged due to aluminium toxicity cannot explore more volume of soil limiting the uptake, transport and utilization of nutrients and hence the concerned plants become more vulnerable to mineral nutrient deficiencies and drought (6,8-11), which ultimately leads to food security threat. Aluminium is among one of the major constituents of the earth's crust and highly acid soils constitutes approximately 30% of the earth's total land area (12). Under acidic conditions, soil minerals releases aluminium as $Al(OH)^{2+}$, $Al(OH)_2^{+}$ and $Al(H_2O)^{3+}$ (4). Plants rapidly takes up the aluminium available in acid soils leading to chemical stress. Aluminium content in cultivated plants is higher in roots. Shoots accumulate very less concentration of aluminium (13-14). The mechanism of transport is not fully understood (15). Yet in the central region of Brazil a concentration of aluminium on the leaves of some wild species of "cerrado", can be observed (16-17). Indeed aluminium toxicity is the primary limitation for production of many crops on acid soils. Soil acidity can be reduced by raising soil pH through application of lime but it is not practical and cost effective.

The aluminium solubility is pH dependent. Lime application can be used for reducing aluminium toxicity by raising soil pH. Liming of acid soils results in formation of hydroxy-aluminium precipitates which in turn reduces the soluble and exchangeable aluminium to lower or almost negligible levels hence limiting the toxicity effects on crop growth and yield. However this amendment does not remedy subsoil acidity, and liming may not always be practical or cost-effective. To combat aluminum toxicity in soil, a microbial based approach has been followed. Microbial inoculants has great potential to confer resistance to these toxicities by means of production of chelating compound like siderophores, organic acid and exopolysaccharides that binds heavy metal ions making them unavailable to plant thereby reducing their toxic effects.

Materials and Methods

In vivo experiment

A Pot Experiment was conducted at Indian Agricultural Research Institute, PUSA, New Delhi. Maize seeds were grown in soils having 60mM $AlCl_3$ stress. Soil used for conducting this experiment was collected from IARI, New Delhi farm. The soil used was a sandy loam, mixed and moderately permeable. Soil was made acidic by adding aluminium chloride (60mM). Seeds were coated with Carboxy Methyl Cellulose (CMC) before sowing. Some treatments were given to seeds with CMC as follows: 1. inoculated by *Burkholderia* culture 2. inoculated by *Bacillus* culture 3. control without inoculants. For comparative study these treated seeds were also sown in regular IARI soil which was not given any external aluminium stress. Watering was carried regularly and plants were allowed to grow for 13 days.

Harvest

Plants were harvested from pots on 13^{th} day after sowing. Roots were gently washed with double distilled water in order to eliminate soil particles adhering to its surface. The length of the roots and plant height were measured with a milimetric rule.

Digestion of samples

Root samples were digested for phosphorus and aluminium content measurement. 0.1 g of root sample from each treatment was grinded separately and 10ml of di-acid mix containing nitric acid and perchloric acid in the ratio 9:4 was added to each sample and kept for overnight incubation. Further incubation was carried on hot plate for 1 hour. Crystal clear solution is obtained. The solution is cooled and 10ml distilled water was added. Samples were filtered with whatman filter paper in volumetric flask and volume was made upto 50ml.

Phosphorus estimation in plant tissues

This was done by ascorbic acid method described by Murphy and Riley (1962) (18). The assay principle is as follows: In acid medium, the reagent containing Ammonium Molybdate and Potassium Antimony Tartarate reacts with orthophosphates to form heteropoly acid named phosphomolybdic acid, which is finally reduced by ascorbic acid to intensely colored molybdenum blue and the intensity of this blue colored solution was measured at 660nm using UV Visible spectrophotometer.

Aluminium determination

Aluminium concentrations in root and shoot tissues were determined separately by using aluminon-acetate buffer (Barnhisel and Bertsch, 1982) (19). 5ml aliquot was taken in a volumetric flask and volume was made to 10ml with the help of distilled water. To this solution, 0.5ml 0.5% ascorbic acid was added. The solution was kept in water bath for 30 minutes at 90^0C. Finally the solution was cooled to room temperature and 5ml aluminon acetate buffer was added. The absorbance was thus measured at 530 nm in a UV Visible spectrophotometer.

Results

Plant height and Root length

The current study depicted that under aluminium stressed conditions there was a decrease in overall plant height and root length but bacterial inoculation was found to be much beneficial in combating this toxicity effect in roots. The seeds inoculated with *Bacillus* and *Burkholderia* inoculums increased the root length significantly by 1.4- folds and 2- folds respectively thereby combating the effect of aluminium toxicity but unfortunately such promising results were not found in case of plant height (Fig. 1).

Aluminium uptake and phosphorus solubilisation in roots at 13th day of inoculation

Aluminium concentration was found maximum in roots of plants which were grown under aluminium stress condition. But this aluminium accumulation decreased ~2-folds when *Burkholderia* was used as seed inoculants under aluminium stress conditions. However *Bacillus* didn't show such significant results (Fig. 2).

Phosphorus solubilisation in roots was increased upto 30% on *Burkholderia* inoculation at 60mM aluminium accumulation. However, in this case also *Bacillus* inoculation didn't show any significant difference (Fig. 3).

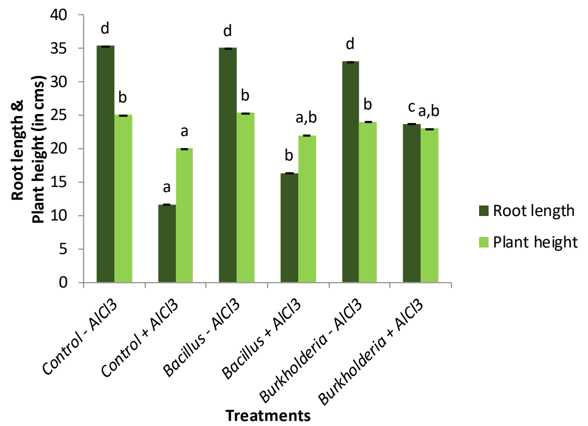

Figure 1. Root length and plant height at 60mM aluminium concentration.

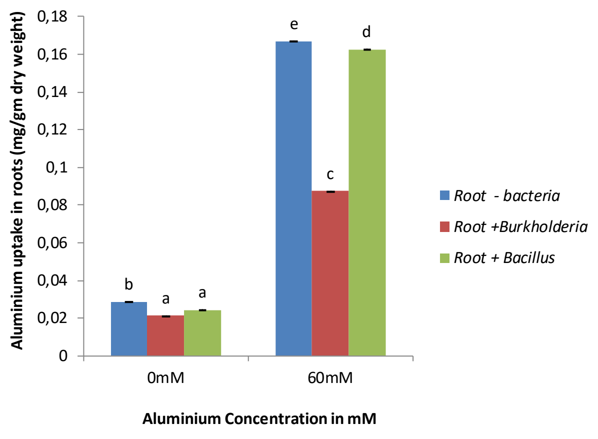

Figure 2. Aluminium accumulation in roots with different inoculum treatments at 60mM aluminium concentration.

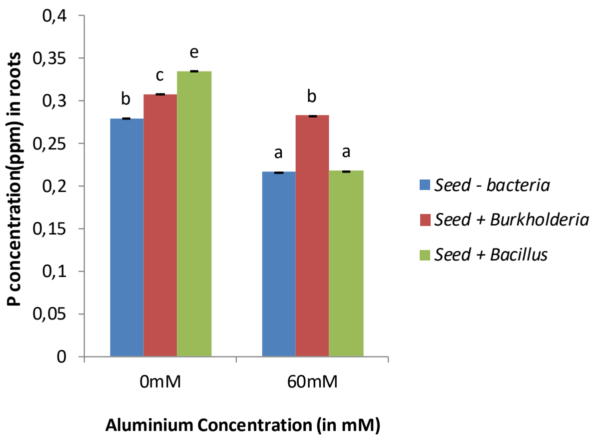

Figure 3. Phosphorus solubilisation in roots with different inoculum treatments at 60mM aluminium concentration.

Discussion

A high degree of correlation was found in aluminium toxicity and growth of roots indicating a predominant role of aluminium toxicity in root growth depression (6, 20). The growth of maize root was significantly reduced when they were allowed to grow in soils having aluminium toxicity. Hairiah *et al* reported a reduction in principal root length of *Mucuna pruriens L.* and *Mucuna deeringiana* L. under aluminium toxic conditions (21). Keltjens and Dijkstra also observed the same in case of *Triticum aestivum L.* (22).

The aluminium concentration was also found higher in roots grown under aluminium toxic conditions indicating aluminium accumulation in roots. This has also been reported previously (13, 21, 23).

But the aluminium accumulation in roots was found to be reduced when seeds were grown with the aid of bacterial inoculants and hence proved beneficial in root growth and development. The current observation has also been supported by previous citations where inoculation with PGPRs reduced salinity stress in lettuce plants (24). *Pseudomonas aeruginosa* was also found responsible for providing tolerance against Zn stress in wheat (25). Appanna also reported the role of *Pseudomonas fluorescens* in tolerating aluminium toxicity via aluminium detoxification through extracellular lipid compound production (26).

Barker and Pilbeam also explained the reduction in root cell division thereby inhibiting the elongation of roots (27). *Spingomonas* spp. has been reported previously for synthesizing exopolysaccharides (28) and hence providing protection to to cell by forming a boundary (29). These exopolysaccharides are responsible for chelating these heavy metals (30). From the current findings use of Burkholderia isolates as seed inoculants could be suggested in sequestering aluminium toxicity in maize crops.

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