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RESEARCH ARTICLE

EVALUATION OF TOLERANCE LEVEL OF *BURKHOLDERIA* STRAIN AGAINST ALUMINIUM STRESS AND ITS ROLE AS A BIONOCULANT ON CHLOROPHYLL CONTENT AND NITRATE REDUCTASE ACTIVITY IN *zea mays*

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ABSTRACT

Aluminium is one of the most abundant elements on earth's crust. It forms approximately 7% of the earth's crust. In soils having low pH, aluminium combines very frequently with oxygen forming complexes which are rather more stable and harmful to microbes as well as plant species. Microorganisms continuously interact with various elements present in soil. Some interactions are beneficial but under low pH conditions some interactions are there which can cause inhibitory effect on them thereby limiting their growth under such conditions. Our current study aimed at studying the tolerance level of *Burkholderia* strain under aluminium toxic conditions and its role as bioinoculants on chlorophyll content and nitrate reductase activity of leaves in *Zea mays*. The results clearly depicted that *Burkholderia* species were able to survive even at 20mM aluminium stressed conditions and further when this strain was used as bioinoculant for coating maize seeds under stressed conditions, the plant thus grown resulted in an increase in chlorophyll content as well as nitrate reductase activity by approximately 1.2- folds and 2.5- folds respectively. The research revealed that *Burkholderia* has immense potential to tolerate stress thereby enhancing physiological parameters of plants. Further, elaboration of this study may explore new insights towards mechanism involved and the role of microbes in improving crop productivity.

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INTRODUCTION

Heavy metals are most probable source of introducing pollution into the environment. They accumulate in the environment and lead to hazardous effect on both flora and fauna (Mishra *et al*., 2008). Heavy metals are indeed responsible for contaminating majority of the agricultural soils around the world resulting in soil acidity. Acidic soils are prevalent in approximately 40% of the world's arable soils posing a range of toxicity threats to surrounding species (Uexküll *et al*., 1995). The major cause behind this may be the prolonged use of phosphate based fertilizers, irregular or inefficient watering of land, industrial wastes, etc. (Passariello *et al*., 2002; Schwartz *et al*., 2001; Bell *et al*., 2001, Sani, 2001).

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The contaminated soil thus results in reduced crop yield, affecting sustainable agriculture (Lin *et al*., 2005; Molas, 2002 Horst *et al*., 2010; Sun *et al*., 2007; Foy *et al*., 1978).In the earth's crust, aluminium is one of the most copious heavy metal (Darko *et al*., 2004). Free aluminium ions are taken up from the toxic soils (Borkowska, 1988). Growth of major agricultural crops is limited under such acidic conditions and plants however grown show impaired growth and development (Maslowski, 1997). Under stressed conditions, epidermal layer of major plant parts gets damaged due to loss in their turgidity (Wagatsuma *et al*., 1987). Necrosis, calcium and phosphorus deficiency are the major symptoms of aluminium toxicity. Microorganisms are present in abundance in soil where they continuously interact with various inorganic ions present. Some interactions prove beneficial for microbes while others pose inhibitory effects on them by hindering their metabolic activities. This toxicity stress in soil may result in selection of tolerant microbes that are capable of surviving under stressed conditions (Trevors *et al*., 1986; Belliveau *et al*., 1987;

Trevors, 1987; Silver and Walderhaugh, 1992; Slawson *et al*., 1992). These microbes can be utilized as bioinoculants thereby enhancing the physiological activities of plants by suppressing the effect of aluminium toxicity. The current study was aimed at estimating bacterial growth against aluminium tolerance and role of these inoculants in improving physiological traits of maize plants especially chlorophyll content and nitrate reductase activity.

MATERIALS AND METHODS

Microorganisms and their culture

Burkholderia isolates were evaluated for their tolerance to 20mM aluminium stressed conditions. Screening was carried out on nutrient broth fortified with 0mM and 20mM aluminium concentration. The prepared supplemented medium was dispensed in tubes. The experiment was carried out in triplicates. The *Burkholderia* strain was then inoculated aseptically in the medium in laminar air flow. The inoculated media was incubated on rotatory shaker at 37°C for 24-48h. Optical density was then measured at 600nm to estimate the tolerance of *Burkholderia* strain to 20mM aluminium concentration.

Total chlorophyll content

The seeds of *Zea mays* were inoculated with *Burkholderia* strain and grown in aluminium stressed conditions. Total chlorophyll content was then estimated for such plant grown in stress. The estimation of chlorophyll content was carried out as per Arnon method (1949). The leaf samples (0.5g) from all the treatments (control without $AlCl_3$, control with $AlCl_3$ and inoculated plant in $AlCl_3$ condition) were crushed with 80% chilled acetone (20ml) in mortar and pestle individually. The extract is then filtered with whatman filter paper no. 1. Absorbance was recorded at 645 and 663nm after making the final volume to 100ml with 80% acetone.

Nitrate reductase activity assay

Nitrate reductase activity was determined by the method described by Klepper *et al*. (1971) with few modifications. Incubation solution containing 0.1 $mmol.L^{-1}$ potassium phosphate buffer pH 7.5, 0.05 $mol.L^{-1}$ KNO3 and 1% v/v isopropanol was prepared. Leaf discs with 5mm diameter weighing approximately 100mg were vacuum infiltrated in 2ml of this incubation solution for 1hr at 30°C in the dark. For nitrate reductase activity estimation, 1 mL each of 1% sulfanilamide in 1 mM L^{-1} HCl and 0.02% naphthyl ethylenediamine dihydrochloride (NEDD) were added. The test tubes were vortexed and absorbance was recorded at 540 nm with a Double beam UV-VIS spectrophotometer (UV5704SS).

RESULTS AND DISCUSSION

Burkholderia strain was evaluated for its tolerance to 20mM aluminium stress and it showed positive results and moreover the growth was better in aluminium supplemented media. The results clearly depicted that acidic medium is favorable for *Burkholderia* strain (Table 1). According to previous studies bacterial isolates have been found to show their growth promoting potential. Earlier growth promoting potential of bacterial isolates has been reported at 2071µM and 3106µM aluminium concentration respectively (Parmeela, 2002). The capacity of *Burkholderia* isolates to survive under aluminium stressed condition can be utilized in enhancing physiological parameters of various important crop species. Many of the plant species growth get hampered due to aluminium toxicity as aluminium in the form of phosphate gets precipitated in the roots (Barlett & Reigo, 1972) and inhibits cell division in roots and shoots by binding to nuclear DNA. Also, energy metabolism is severely affected under stressed condition (Bennet& Breen, 1993). As expected the unstressed plants showed highest level of chlorophyll content but a decrease of approximately 1.2- folds was found in stressed conditions. Similarly nitrate reductase activity was also decreased by 1.6- folds in toxic conditions. However when *Burkholderia* was applied as inoculants to maize seeds, the chlorophyll content and nitrate reductase activity showed increase of 1.2-folds and 2.5- folds respectively (Fig. 1 and 2).

Table 1. Growth of *Burkholderia* strain in 20mM aluminium stress

Aluminium concentration (in mM)	*Burkholderia* growth
0	0.78 ± 0.003
20	0.96 ± 0.027

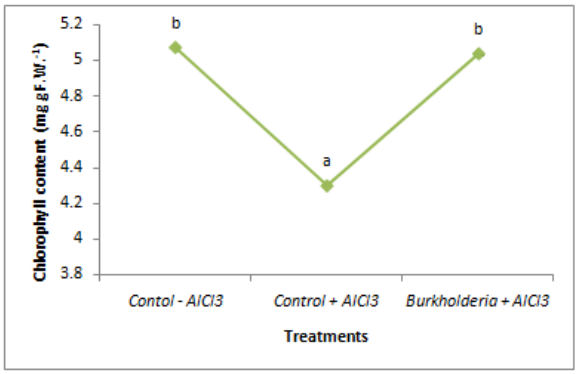

Fig. 1. Influence of *Burkholderia* inoculum on chlorophyll content of maize leaves from plants grown under aluminium toxic conditions

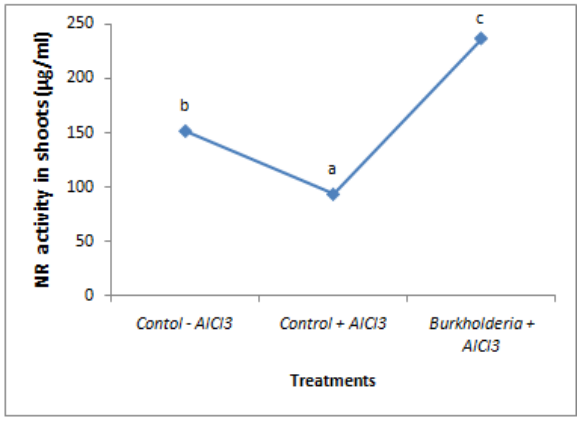

Fig. 2. Influence of *Burkholderia* inoculum on nitrate reductaseacivity of maize leaves from plants grown under aluminium toxic conditions

This reduction in chlorophyll content can be one of the major causes of low photosynthetic rate in aluminium toxic conditions (Okhi, 1987). It has also been reported that several metabolic processes are also inhibited in aluminium toxic conditions which inturn leads to lowering of chlorophyll content in affected plants (Aniol, 1981; Marschner, 1995). The current study revealed the role of bacterial strains in lowering aluminium toxic effects in terms of certain physiological parameters. Further studies could eventually lead to exploration of aluminium sequestering strategies adopted by microbes and understanding of their role in beating aluminium toxicity effects in plant species in a more broad conduct which may drastically improve crop development and productivity.

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