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Exploring the role of *Bacillus species* in combating Aluminium toxicity in *Zea mays* by studying its effect on chlorophyll content

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Abstract: *Aluminium is one of the major components that are present in soil and it forms complexes with oxygen and silicates. These complexes are stable and rapidly soluble in soil water under low pH conditions. Some microorganisms present in soil have great potential to survive under these acidic conditions. The present study was aimed to check the effect of aluminium toxicity on Bacillus species when their growth media was supplemented with 20mM $AlCl_3$ concentration. Experimental results depicted that Bacillus had best survival at 20mM $AlCl_3$ concentration. Surprisingly it showed an increase of ~ 80% in bacterial growth under 20mM aluminium toxic conditions as compared to unstressed condition, implying that Bacillus species which were isolated from soil have growth optima at low pH values. When this combination was applied to germinate maize seeds in acidic conditions , the maize seeds showed ~ 15% increase in chlorophyll content of their leaves in comparison to the control leaves without any bacterial inoculation under stressed conditions. In the light of the findings, it can be concluded that the problem of aluminium toxicity can be addressed efficiently by using microbes and there can be significant enhancement of growth parameters as well.*

Key words: *Bacillus, Zea mays, Aluminum, chlorophyll, Toxicity*

I. Introduction

Heavy metals are generally naturally occurring in the earth's crust but now-a-days various anthropogenic sources viz., industrial effluents, mining, dumping of sludge are found to be responsible for introducing these heavy metals to environment on regular basis [1]. Heavy metal in present era forms an important class of pollutants. Their retention in the environment exerts hazardous effects both on flora as well as fauna [2]. It has been found that higher concentration of these heavy metals have become one of the major limiting factor for production of crops leading to a loss in sustainable agriculture [3,4]. These heavy metals may interact with active sites of enzymes and nucleic acids thereby leading to cell death [5,6,7].

Acidic soils contribute to almost 40% of the world's arable soils presenting various toxicity hazards to various species [8]. Increase in the concentration of heavy metals may adversely affect metabolic activities of various microbes. Aluminium is one of the most abundant heavy metals that is found in earth's crust contributing approximately 7% [9]. It has been found to have various negative effects on growth and development of major crops in acid soils [10,11,12]. Plants take up free aluminium ions from acidic soils [13]. Also, under circumstances where aluminium concentrations are higher in soil, the above ground parts of plants are found to have impaired development [14]. The epidermal cells lose their turgidity due to changes in their cell membrane permeability thereby damaging the epidermal layer of major plant parts [15]. Necrosis of leaves has been found under aluminium toxic conditions. The phosphorus and calcium levels also lowers down in presence of aluminium thus leading to death of shoot tips.

The aim of present research was to study the tolerance level of bacterial cultures under aluminium toxic conditions and to study the combined effect of bacterial *spp.* and aluminium toxicity on total chlorophyll content of leaves grown under control as well as stressed condition.

II. Materials and Methods

Microorganisms, Growth medium and Culture conditions

Bacillus spp. resistant to high aluminium concentration was isolated from north east soil (soils with acidic pH). Isolation was carried out on nutrient broth fortified with different concentrations of aluminium (0, 20, 60, 80mM $AlCl_3$). The fortified medium was dispensed in tubes in triplicates. The *Bacillus spp.* was then inoculated aseptically in the medium in laminar air flow. The inoculated media was put on shaker for 24-48 hrs at 37°C and then O.D. was taken at 600nm to check aluminium tolerance of inoculated *Bacillus spp.*

Determination of total chlorophyll content

Maize seeds inoculated with selected *Bacillus spp.* were grown in soil bearing aluminium stress. The leaves from such plants were taken to estimate variation in total chlorophyll content under aluminium stressed conditions. The chlorophyll estimation was carried out as per Arnon method [16]. 0.5g of leaves from control plants (without $AlCl_3$), stressed plants (with $AlCl_3$), and stressed plant with *Bacillus spp*. inoculation were homogenized separately with mortar and pestle in dark in 20ml of 80% chilled acetone. The extract filtration was then carried out with the help of Whatman filter paper no. 1. The final volume was made with 80% acetone upto 100ml. Absorbance was then recorded at 645nm and 663nm respectively.

III. Result and Discussion

Aluminium toxicity was checked on solid media for *Bacillus species*. It was found that *Bacillus spp.* was tolerant upto 20mM $AlCl_3$ concentration. Beyond this, the tolerance level declined steeply and no bacterial growth was observed above 20mM $AlCl_3$ concentration and more interestingly the *Bacillus* showed more growth at media supplemented with 20mM $AlCl_3$ concentration as compared to non-supplemented media suggesting that slightly acidic pH is favorable for *Bacillus* growth (Fig. 1).

The growth promoting potential for some bacterial isolates has been reported previously also at aluminium concentrations of 2071µM and 3106µM respectively [17]. The aluminium tolerance capacity of *Bacillus spp.* makes it an excellent bioinoculant for production of crops under aluminium toxic conditions.

In soils with acidic pH, aluminium gets precipitated in phosphate form either in the root walls or root surface thereby hampering development of roots in many crops. This may be due to unavailability of soluble phosphorus [18]. Aluminium binds to nuclear DNA and inhibits cell division in root and shoot tips. Respiration is also affected due to this toxicity which interferes with the energy metabolism of plants [19].

The average higher chlorophyll content was found in plants which were grown in unstressed condition. The chlorophyll content of leaves decreased approximately 15% under aluminium stressed condition. But when the seeds were inoculated with *Bacillus spp.* and then grown under aluminium toxic condition, there was on an average negligible decrease in chlorophyll content of leaves (Fig. 2). Chlorophyll content of leaves decreased due to aluminium toxicity which in turn was responsible for lowering photosynthesis rate [20].

It has also been observed that aluminium toxicity and deficit of different elements observed in presence of Al^{3+} inhibits several metabolic processes involving enzymatic activity and nucleic acid synthesis which may thus lead to decrease in chlorophyll content of leaves [21,22]. From the current study, it could be suggested that bacterial cultures if used as seed inoculants could prove to be a boon in sequestering aluminium toxicity thereby enhancing growth and development of many important crop species.

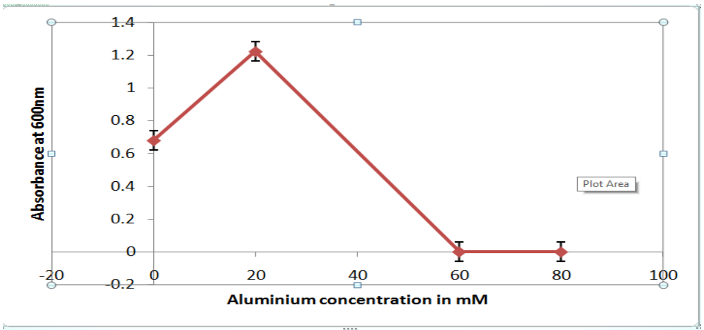

Fig. 1. Influence of Al^{3+} concentration on growth of *Bacillus species*

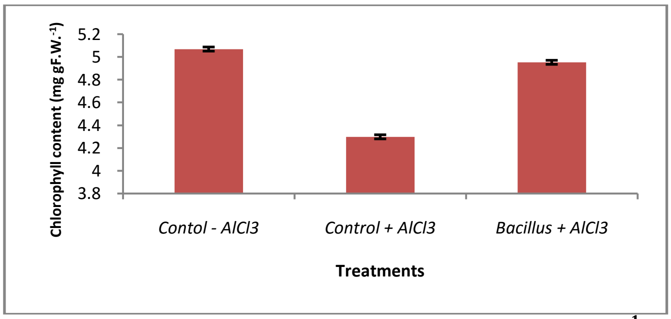

Fig. 2. Influence of *Bacillus* inoculum on chlorophyll content (mg $gF.W.^{-1}$) of plants grown under aluminium stressed conditions

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References

[1]. Sani R K., Copper-induced inhibition of growth of Desulfovibrio desulphuricans G20: Assessment of its toxicity and correlation with those of Zinc and Lead. Appl Environ Microbiol., 2001, 67: 4765-4772.
[2]. Mishra V K and Tripathi B D., Concurrent removal and accumulation of heavy metals by the three aquatic macrophytes. Bioresour. Technology, 2008, 99: 7091-7097.
[3]. Lin S C, Ye G W, Wu J Z, Zheng Y B and Lin W X., Studies on the Comparison of the Chemical Compounds in Lotus Seeds (Nelumbo nucifera Gaertn.) Between "Tai-Kong lotus 36" and "Jian lotus". Strait Pharmaceutical Journal, 2005, 17 (4): 91–93.
[4]. Molas J., Changes of chloroplast ultrastructure and total chlorophyll concentration in cabbage leaves caused by excess of organic Ni II complexes. Environ. Exp. Botany, 2002, 47: 115-126.
[5]. Ohsumi Y., Changes induced in the permeability barrier of the yeast plasma membrane by cupric ion. J Bacteriol, 1998, 170: 2676-2682.
[6]. Hazel J R & Williams E., The role of alteration in lipid composition in enabling physiological adaptation of organisms to their physical environment, Prog Lipid Res, 1990, 29: 167-227.
[7]. Cervantes C & Gutierrez-Corana F., Copper-resistance mechanism in bacteria and fungi. FEMS Microbial Rev., 1994, 14: 121-137.
[8]. Uexküll HR, Mutert E., Global extent, development and economic impact of acid soils. Plant Soil, 1995, 171: 1–15.
[9]. Darko E, Ambrusa H, Stefanovits-Banyai E, Fodor J, Bakos F & Barnabas B., Aluminium toxicity, Al tolerance and oxidative stress in an Al-sensitive wheat genotype and in Al-tolerant lines developed by in vitro microspore selection. Plant Sci., 2004, 166: 583-591.
[10]. Horst WJ, Wang Y, Eticha D., The role of the root apoplast in aluminium-induced inhibition of root elongation and in aluminium resistance of plants: a review. Ann. Bot., 2010, 106: 185–197.
[11]. Sun P, Tian QY, Zhao MG, Dai XY, Huang JH, Li LH, Zhang WH., Aluminum-induced ethylene production is associated with inhibition of root elongation in Lotus japonicus L. Plant Cell Physiol., 2007, 48: 1229– 1235.
[12]. Foy C D, Chaney R L, White M C., The physiology of metal toxicity in plants. Ann. Rev. Plant Physiol., 1978, 29: 511–566.
[13]. Borkowska B., Toksycznosc glinu (Al). Wiad. Bot., 1988, 32: 157-166.
[14]. Maslowski J., Zmiennosc cech biometrycznych u genotypow pszenicy jarej tolerancyjnych na toksyczne jony glinu. Biul., 1997, IHAR 203: 57-61.
[15]. Wagatsuma T, Kaneko M. & Hayasaka Y., Destruction of plant cells by aluminium. Soil Sci. Plant Nutr., 1987, 33: 161-175.
[16]. Arnon D I., Copper enzyme polyphenoloxides in isolated chloroplast, 1949, 24 : 1-15.
[17]. Parmeela., 'P' Solubilization potential of aluminium tolerant growth promoting fluorescent pseudomonads. Asian J Microbial Biotech Environ Sci., 2002, 4: 381-383.
[18]. Barlett R J & Reigo D C., Effect of chelation on the toxicity of aluminium. Plant Soil, 1972, 37: 419-423.
[19]. Bennet R J & Breen, C M., Aluminium toxicity: towards an understanding of how plant roots to the physical environment. Genet. Aspect Plant Min. Nutr., 1993, 103-116.
[20]. Okhi K., Aluminium stress on sorghum growth and nutrient relationships. Plant Soil, 1987, 98: 195-202.
[21]. Aniol A., Metody okreslania tolerancyjnosci zboz na toksyczne dzialanie jonow glinu. Biul., 1981, IHAR 143: 3-14.
[22]. Marschner H., Mineral Nutrition of higher plants., 1995, Academic Press, London.