Biochemical analysis and Antioxidant activities of Spinach by Plant Growth Promoting Bacteria (PGPB) under arsenic stress

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ABSTRACT

Spinacia oleracea L. commonly known as spinach is vegetable crop used throughout the world and accumulates heavy metal from rhizosphere soil. Arsenic is a toxic heavy metal usually found in soil and water which causes harmful effects on plant growth and development and carcinogenic for human. The present study was aimed to enhance plant growth of spinach by reducing the hazardous effects of arsenic following the use of Plant Growth Promoting Bacteria (PGPB). Sterilized seeds were soaked in PGPB inocula 3-4h prior to sowing and grown in pots under natural condition. The plants were irrigated with arsenic contaminated water. The results demonstrated significant decrease in growth as compared to control while the PGPB treatment exhibited an increased growth even in presence of arsenic as compared to control. The chlorophyll a (41.92%), chlorophyll b (66.34%), carotenoids (28.92%) proline (44.19%), protein (27.35%), phenolics (29.62%), flavonoids (20.11%) and sugar (44.31%) contents were significantly enhanced by the treatment of PGPB. The antioxidant and defense related enzyme activities viz. Catalase (CAT), Phenylalanine ammonia-lyase (PAL), Polyphenol oxidase (PPO), Superoxide dismutase (SOD) and Peroxidase (POD) activity were greater by 64.95%, 41.1%, 59.45%, 25.58% and 22.85 % respectively in PGPB treated plants. It is inferred that plant growth promoting bacteria is an efficient tool to reduce the oxidative stress and induce defense potential of plant in addition also increase plant growth and nutritive qualities.

Keywords: Arsenic, Biochemical parameters, PGPB, Antioxidant enzymes, Spinach

INTRODUCTION

Spinacea Olearacea L. (Spinach) Commonly known as, Palak belong to Amaranthaceae family is a leafy vegetables which contains high vitamin contents such as carotene, vitamins (A, B, B1, B2, B3), minerals Zinc, Magnesium, Iron and Calcium) and folic acid. (Liu et al., 2021). Spinach is cultivated throughout the world due to its rapid growth rate, increased biomass production and use of heavy metals and other important nutrient from soil. (Zaheer *et al*., 2020).

Arsenic is a 20th natural metalloid in the ground water, is a global threat for humans as it can lead to many disorders of skin, vascular and nervous systems as well as cancer (Podgorki and Berg, 2020). Arsenic accumulation adversely affects the plant growth due to its toxicity, mobility, and persistence it reduces the crop quality and yield and thereby serious threat to food safety and security (Shahid *et al*., 2020).

Plant Growth Promoting Bacteria (PGPB) play a vital role in biodegradation of heavy metals and stimulate plant growth in the presence of heavy metals. Bioremediation of heavy metals can be accomplished by

various mechanism such as phytoextraction, rhizofiltration, phytovolatilzation and phytostabilization (Mazhar *et al*., 2020).

OBJECTIVES

The present Study aims to observe the effect of PGPB on spinach growth under arsenic stress

MATERIAL AND METHODS

Seeds (Spinach local var.) were surface sterilized with the 95% of ethanol for 5 minutes and successively washed with autoclaved distilled water. Prior to sowing the seeds were soaked in PGPB inocula for 4 hours. The plants were grown under natural conditions. Following treatments were made. Control (Untreated Seeds plants irrigated with tap water), T1 (untreated seeds plants irrigated with arsenic polluted water, T2 (PGPB inoculated seeds and irrigated with arsenic polluted water). Plants were irrigated on alternate day of arsenic (55µg/L) contaminated tap water.

Biochemical Analysis

Method described by Bates et al., 1973 was followed to calculate proline content. (Proline content (mg / g) = K value × dilution factor × Absorbance (O.D) /weight of the sample). Method described by Singleton and Jones, 1999 was followed to measure the phenolic contents. To measure the flavonoids content the Zhishen et al., 1999 protocol was followed. Sugar content was measured by using the protocol of Dubois et al., 1956 while Protein content was measured by using the protocol of Lowery et al., 1951. Protein content (mg / g) = K value × Absorbance × Dilution Factor/ Sample weight (K value = 19.6). Chlorophyll a, b and crotonids were calculated by following the protocol of Armon, 1949 and using following formulas.

Chlorophyll a = 12.7 x OD663 -2.69 x OD645

Chlorophyll b = 22.9 x OD645 - 4.68 x OD663

Carotenoids = 4 × OD × Total sample vol. / fresh weight of Spinach leaves.

Antioxidant Enzyme Activity

Phenylalanine ammonia -lyase (PAL) activity was measured by using the protocol of Suzuki *et al.*, (2003). Polyphenol oxidase (PPO) activity was measured by using the protocol of Myer *et al*., (1974). The protocol of Fridovich, 1976 was followed to measure the Superoxide dismutase (SOD) activity. Peroxide (POD) activity was measured by using the protocol of Vetter et al., (1958). Catalase (CAT) was measured by following the protocol of Kumar *et al.*, (2010).

RESULTS

Figure **1** demonstrated that Arsenic causes the decrease in growth, physiological parameters of spinach. PGPB showed a significant improved result as compared to control group. PGPB also decreased the negative impacts of arsenic and showed positive results. The chlorophyll a (41.92%), chlorophyll b (66.34%) and carotenoids (28.92%) were significantly enhanced. The proline (44.19%), protein (27.35%), phenolics (29.62%), flavonoids (20.11%) and sugar (44.31%) contents were also enhanced.

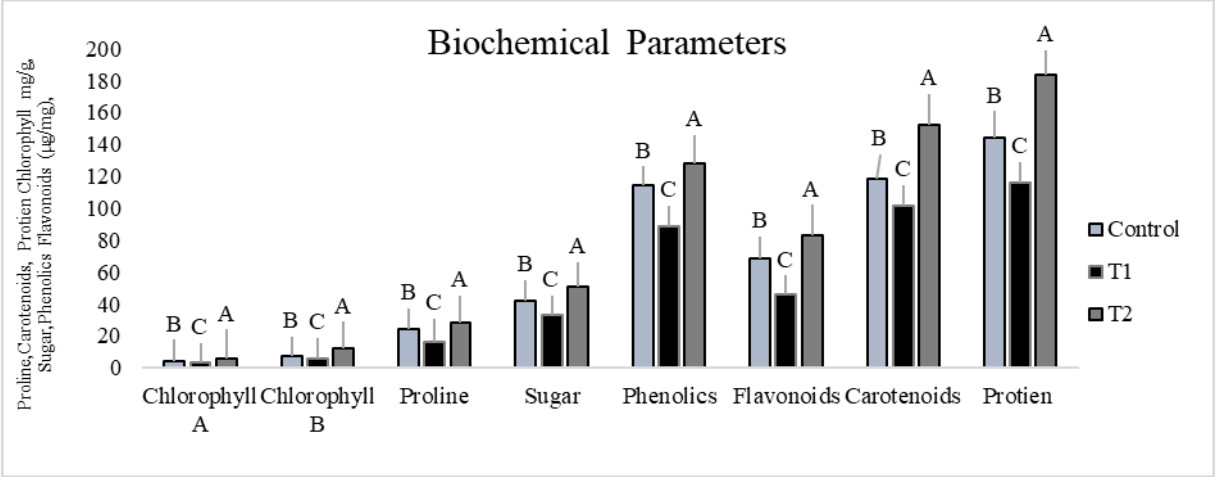

Figure 1. Effect of arsenic and PGPB on Biochemical Parameters.

The Bars on graph represents the standard error and the values followed by different letters are significantly different. Statistical comparison was computed at P≤ 0.05 by LSD test using software statistics 8.1 version. LSD value for Chll a & b = 0.03, Proline= 1.72, Sugar=1.71, Phenolics = 1.50; Flavonoids = 1.77; Carotenoids= 1.41; Protein= 1.91. (T1= Arsenic treated plants, T2= PGPB+ arsenic irrigation)

Antioxidant Enzyme Activities

Figure 2 demonstrated that antioxidant enzyme activities viz. Catalase (CAT), Phenylalanine ammonia-lyase (PAL), Polyphenol oxidase (PPO), Superoxide dismutase (SOD) and Peroxidase (POD) activity were enhanced by 64.95%, 41.1%, 59.45%, 25.58% and 22.85 % respectively in PGPB treated plants.

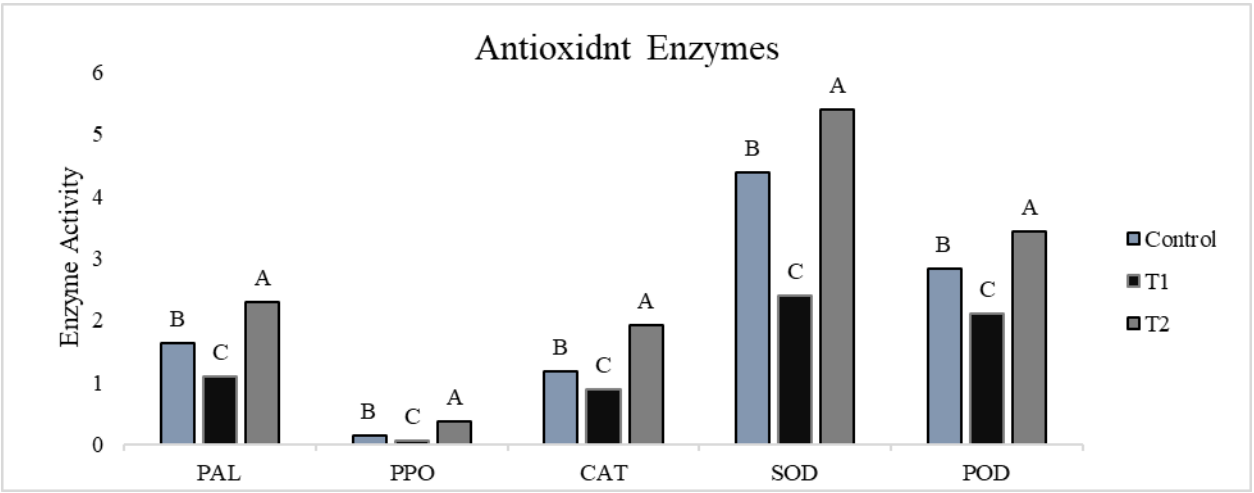

Figure 2. Effects of arsenic and PGPB on Antioxidant Enzyme Activities

The Bars on graph represents the standard error and the values followed by different letters are significantly different. Statistical comparison was computed at P≤ 0.05 by LSD test using software statistics 8.1 version. LSD value for PAL = 0.17; PPO = 0.01; CAT= 0.01; SOD= 0.05; POD= 0.01 (T1= Arsenic treated plants, T2= PGPB+ arsenic irrigation)

CONCLUSION

From the present study it is concluded that arsenic causes the decrease in plant growth and biochemical parameters of spinach as well as antioxidant enzyme activity. PGPB enhances Biochemical parameters and antioxidant enzyme activities in the presence of arsenic. PGPB is an ecofriendly and efficient tool for the agriculture to improve the crop productivity.

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