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Research article

Effect of NaCl and glyphosate on root exudates of Cajanus Cajan plant

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ABSTRACT

Plants communicate with neighboring plants and other organisms surrounding them. Aboveground communication is articulated through stems, leaves, or flowers while below- ground communication is mediated by roots. The plant root is capable of secreting chemicals into the rhizosphere through root exudates. The chemical constituents of the root exudates are characteristic of a particular plant species and also depend on the surrounding biotic and abiotic environment. Recent research suggests that the root exudates act as a sort of chemical 'language' between the secreting plant and other organisms in the rhizosphere.

An experiment was conducted in axenic condition to study the effect of salt and weedicide (glyphosate) on Rhizobium sp. of Cajanus cajan plant. Total seven carbon sugars and 16 amino acids were determined from N, Ng, H & Hg plants treated with NaCl and Glyphosate. H and Hg produced highest amount of sucrose, fructose & mannose also glycine, proline and isoleucine were detected in the root exudates of H & Hg on 10th and 15th day. However it concludes that stress enhances production of sugars and amino acids in root exudates.

Keywords: Root exudates, stress, Rhizobium sp., Cajanus cajan, weedicide.

INTRODUCTION

Pigeon pea [Cajanus cajan (L) Millsp] is legume pulse crop, source of protein and constitute an integral part of diet. Now day's the yield of the crop is low due to several limiting factors such as various biotic & abiotic stresses. Among abotic stresses that limit crop productivity, salinity and drought rank as the most detrimental, soil salinity adversely affect plant growth and also the excessive use of herbicides which enhances the salinity. Although in case of legumes, there are additional problems because it is not only the plant but also the symbiotic Rhizobium bacteria which are sensitive both at free living as well as during the symbiotic relationship. To ascertain the reason for poor growth of plants, different factors responsible for low productivity of crop was investigated. Among the plant factors influencing growth of plants, root exudates are important pre invasive factors which would focus on the research of plants, to know the root physiology and about soil microorganism^[1].

nutrients to the plant roots through mass flow and diffusion, alongside the efflux and accumulation of plant root exudates are a complex mixture of chemicals and organic compounds secreted into the soil by the roots that drive underground interactions. The exact compositions of the exudates are determined by many factors, including species and nutritional status of the plant, soil structure and micronutrient status. Depending on the components secreted by plant roots exudates, they may be able to alter the physical & chemical properties of the soil.

The chemical secreted into the soil by roots are broadly referred to as root exudates. In addition to the classical roles of providing mechanical support and allowing water/nutrient uptake, roots also perform certain specialized rotes, including the ability to synthetic, accumulates and secrete a diverse array of organic and inorganic compounds such as sugars, amino acids, phenol and other secondary metabolites, which had been suggested that they may act as messenger that communicates & initiate biological & physical interactions

These exudates is primarily due to the influence of mineral

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between roots and soil organisms. The chemical secreted into the soil by root serve an important role as chemical attractants and repellants in the rhizosphere, they may regulates the soil microbial community in their immediate vicinity, cope with herbivores, encourage beneficial symbiosis, change the chemical & physical properties of the soil and inhibit the growth of competing plant species, and enhance the possibilities and success of symbiotic relationship. The aim of the study was to determine the carbon sugars and amino acids in root exudates of Cajanus cajan plant grown in different saline and herbicidal conditions ^[2]

MATERIAL AND METHOD

Seed surface sterilization

Seeds of Cajanus cajan plant were sterilized in 70% ethanol for 5 min. Rinsed three times with double distill water. Seeds were grown in Petri dishes lined with filter paper for germination ^[3].

Preparation of inoculums

Four Rhizobium strains N normal condition, Ng (4% glyphosate), H (300 mM NaCl) & Hg (6% NaCl % 300 mM Nacl) were grown in YM broth for 48 h. The bacterial cells were harvested by centrifugation at 12000 rpm for 10 min and washed with 0.85% sterilized phosphate buffer saline. Optical density (OD540) of washed cells were checked and adjusted accordingly. Approximately 10 (9) mL live bacterial cell of N, Ng, H & Hg were used to inoculate four planting unit ^[4].

In vitro growth of N, Ng, H & Hg Cajanus cajan plant

Five days old seedlings were placed on glass tube (2 L x 5 cm) containing 50 ml nutrient sloger solution, the sloger solution was used as nutrient media7, seedlings in each planting unit were

subsequently inoculated with 5 ml of bacterial inoculum. Plants were grown for 25 days in growth chamber with 12 h light/dark cycle at 28oC. Root Exudate collection.

The root exudates were collected from the each planting unit at 10, 15, 20 & 25 days after transplanting. The plants were removed from the glass jars and the plant roots were dipped into distilled water for 1h and after filtration through glass wool, the root exudate solution were kept in glass bottles at -200 C for deterimation of sugars and amino acids.

Sugar Analysis

Sugars were determined using High Performance Liquid Chromatography (HPLC) with refractive index (RI) detector. Galactose, arabinose, Xylose, Fructose and sucrose were determined using NH2 – carbohydrate column. Acetonitrile (75%) was used as mobile phase with a flow rate of

1 ml min-1. Mannose and glucose were determined by using Supel cogel column, phosphoric acid (1%) was used as mobile phase at a flow rate of 0.8 ml min-1.

Amino acid analysis

Amino acid concentration were determined using HPLC by a

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modified method following pre-column derivatisation with AQC reagent (6- aminoquinolyl-N- hydroxysuccinimdyl carbamate, Waters,USA.) ^[5].

RESULT AND DISCUSSION

Exudates collected on 10, 15, 20 & 25th day from the four plants N. Ng. H and Hg grown in different conditions of saline and herbicidal stress of NaCl and glyphosate excluded varieties of component from the roots. The amount and quality of exudates also vary with different stages of development of plants. Not much difference was recorded in the organic compounds collected from exudates, slight increase in carbohydrates content was seen in H & Hg plant. Seven carbohydrates were isolated fructose, galactose, arabinose, glucose, mannose, sucrose & xylose from exudates and their concentration varied in different stress conditions, maximum carbohydrate isolated were galactose, mannose & fructose on 10 &15th day on H & Hg plants. The present study showed that sucrose and fructose were isolated highest in Hg plants on 15th day. The Rhizobium use these root exudates as the energy source for significant nitrogen fixation. Most of the sugars and amino acids concentration were found to be highest within the first two weeks of the growing periods. Similarly, Darrah also reported that the root exudates sugar accumulation was higher during the first two weeks of growth period in wheat seedlings. The root exudates carbon sugars and amino acids concentration showed a decreasing trend at 14 days after transplanting. Similar findings were observed in the rice root exudates grown in hydroponics condition. The decrease rate in exudation of hydroponic culture systems was probably occurred by the accumulation of high levels of organic substances in the root environment that may repressing the release of more organic compounds or by reabsorption of the organic compounds by the plants. In this study lower amount of glucose was detected, this may be due to the plants that were grown in strictly carbon free nutrient solution. In the non-sterile soil system at lower temperature some amount of glucose also could be converted into a water soluble intermediate such as maltose that may be another cause of low glucose accumulation in the root exudates. In the herbicidal stress release of carbohydrates and amino acids from roots by susceptible plants was increased when treated with glyphosate. Increased concentrations of carbohydrates and amino acids resulting from herbicide treatment can profoundly alter the composition and activity of the microbial community in the rhizosphere, also it attribute to root damage and increased internal carbohydrate concentrations leads to enhance exudation of sugars and amino acid ^[6].

There were differences in amino acids production among four plants grown in different conditions. N, Ng, H & Hg grown in presence of NaCl and glyphosate. Highest amount of glycine and proline (184 & 121 µmolg-1) followed by isoleucine on 15th day were found. However on 20th & 25th day slight increase in amino acids were observed as

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compared to 10th day, least amount of tryptophan were recorded on 10th day in all the plants. However, negligible amount of amino acids were recorded on 25th day tryptophan, valine and tyrosine were least.

Amino acids promote the growth of microflora of the rhizosphere .They play a major role in neutralizing the soil pH and altering the microclimate of the rhizosphere through liberation of water and co2, such changes may influence infections of roots by pathogenic fungi, root secretions may play symbiotic or defensive role as plant engages in positive or negative communications between plant, root and M.Os. The exudation of amino acids is frequently enhanced under hypoxic conditions. Based on recent work of M.J. Brimecombe steady increases in the release of carbohydrates and amino acids from roots by

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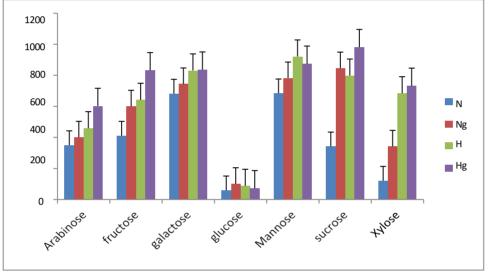
susceptible plants were increased when treated with glyphosate.

Increased concentrations of carbohydrates and amino acids resulting from herbicide treatment can profoundly alter the composition and activity of the microbial community in the rhizosphere

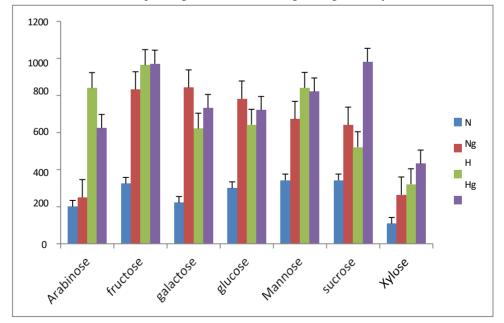
The functional characterization of plant root exudates involved in legume plant has attracted increased attention. The role of root exudates in plant microbial interactions, nutrient acquisition and plant adaptations to environment stress or adverse soil chemical conditions is not only of scientific interest but also implicates obvious practical aspects associated with the need for production of healthy crop plants and for sustainable agricultural systems ^[7].

RESULT OF EXPERIMENTAL DATA

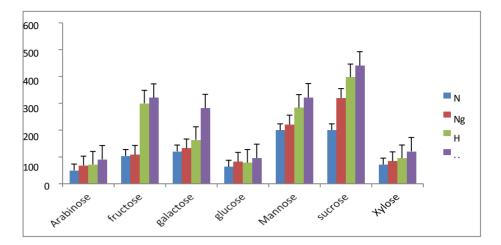




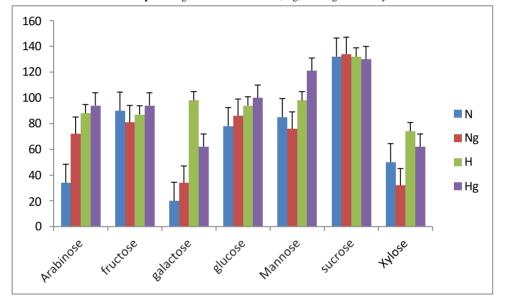
Graph 2: Sugar determined from N, Ng, H & Hg on 15th day



Graph 3: Sugar determined from N, Ng, H & Hg on 20th daY



Graph 4: Sugar determined from N, Ng, H & Hg on 25th day



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