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CHANGES IN ASCORBIC ACID CONTENT IN SUGARCANE AFFECTED WITH *POKKAH BOENG* DISEASE CAUSED BY *FUSARIUM MONILIFORME* SHOLDEN

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ABSTRACT: India, being a mega-diverse country, has extensively cultivated sugarcane (*Saccharum officinarum*) family Gramineae (Poaceae) from ancient to modern times. More than sixty percent of total cane area in the India is in the sub-tropics regions of the country. Gangetic plain of Uttar Pradesh has a fertile area which is highly suitable for agriculture, especially sugarcane cultivation. Sugarcane's production and juice quality is extensively influenced by several parameters having resiliency like weather, variety, cultivation practices and disease causing organisms. Microbial consortium play important role to several changes in sugarcane from early stages till harvest. The aim of the finding work has been focused to point out the modulation in ascorbic acid content in sugarcane affected with *Pokkah boeng* disease caused by *Fusarium moniliforme* Sholden of sugarcane cropping through analysis of regional pattern of its cultivation and level of development of sugarcane. The Pokkah boeng disease (PBD) is a common airborne fungal pathogen *Fusarium fujikuroi* species and adversely affect the yield losses in different way beyond the threshold limit as used varieties of sugarcane throughout the producing countries. The bioactive composition of ascorbic acid in sugarcane juice was assayed by standard procedures to endurance ameliorating the holistic knowledge related to the objective under research.

INTRODUCTION: Sugarcane is a rich source of vitamin C (ascorbic acid). Besides sugar from time immemorial, sailors during voyages used to carry it for chewing purposes and also to prevent the occurrence of scurvy disease, such a valuable crop suffers from several seed-piece transmissible diseases. Culmicolous *Pokkah boeng* *Fusarium moniliforme* Sholden is an important disease, particularly in sub-tropical India ⁹.

It is well defined that ascorbic acid plays a key role as a major non-enzyme antioxidant in plants and mediates certain oxidative stresses caused by biotic and abiotic stress ^{2, 16}. It can enhance the growth of a plant and boost its capacity to withstand stress, and hydroxyproline-containing proteins must be synthesized ⁷.

Under the different agro-practicing systems, agriculture faces many problems with producing seventy percent or more food for the increasing population. At the same time, crop productivity does not increase in tandem with food demand ^{3, 8, 10, 13}. It causes considerable cane tonnage loss and reduces cane quality ^{5, 6, 17}. The multiple regression analysis showed that total soluble sugar content

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was negatively correlated with reducing sugar, soluble protein and superoxide free radical contents. The activities of peroxidases, polyphenol oxidase, superoxide dismutase and Mg^{2+} -ATPase also showed negative correlation with TSS content. The reducing sugar, activities of superoxide dismutase and Mg^{2+} -ATPase were reported to be dominant factors affecting the total soluble sugar content during the course of sugar accumulation, especially analyzed metabolizing enzyme activities in sugarcane¹⁴. The yield of sugarcane is hindered by diseases mostly due to its vegetative propagation through setts, resulting in easy dissemination of causal organism¹⁵. Many researchers have recently used it to determine the primary and secondary metabolites. Additionally, these advanced metabolomics tools have been used in sugarcane tissue culture. Oxidative stress mitigation and initiation of antioxidant and osmoprotectant responses mediated by ascorbic acid in *Brassica juncea* L. subjected to copper (II) stress Ecotoxicol¹². It has been evaluated that the effect of inoculation with three PGPR species in sugarcane reported that the inoculation can play a fundamental role in cultivation, generating great benefits to the crop and saving fertilizers cost for the producers¹⁸. Corn yield and phosphorus use efficiency response to phosphorus rates associated with plant growth promoting bacteria¹⁹.

MATERIALS AND METHODS: An experiment was set up to investigate the interrelationship between drought induced abscisic acid (ABA) biosynthesis and antioxidative defense system and to confer the role of foliar application of ABA in imparting drought tolerance of sugarcane. At the time of different growth stages of the studied variety it has been observed that the occurrence of

increasing the *Pokkah boeng* disease which affects adversely **Fig. 1A, B, C and D**. Generally such disease has been seen in cultivation of different agro climatic region of the country. Due to this disease the quality and quantity of the crop get reduced. The concentration of vitamin-C (ascorbic acid) is adversely affected by the presence of different plant causal organisms. Several workers have reported the changes in an ascorbic acid content in various crops as a result of fungal and nematodes infections^{1, 11}. A part from these is no report in the literature about the changes in ascorbic acid content in Pokkah boeng affected stalks in four susceptible varieties: CoS 98259, CoS 8432, CoS 8436 and CoSe 01424. In connection to study the different parameters the samples from several different stalk parts, viz. leaf (3rd leaf), bud apical meristem and lateral shoots of infected and healthy stalks of four susceptible cane varieties viz. CoS 98259, CoS 8432, CoS 8436 and CoSe 01424 were collected for estimating ascorbic acid. A part from these, the juice from diseased and healthy canes was also examined for ascorbic acid content with standard protocols. The ascorbic acid content was determined by titration method based on reduction of 2, 6-dichlorophenol indophenols dye (Aburg, 1958). Tissues (0.2g) from above stalk parts were dried at $30 \pm 1^\circ\text{C}$ for two consecutive days in an oven, thoroughly ground in 0.4% oxalic acid solution and centrifuged at 4000 rpm for 15 min.

The supernatant was made up to 20 ml by adding more oxalic acid solution. 5ml of the tissue extract was titrated against a standardized indophenols reagent. The appearance of pink colour indicated the endpoint, which persisted for about 15 seconds. Status and symptom of Pokkah Boeng disease in the sugarcane variety Co 0238:



FIG. 1A: CHLOROTIC PHASE I OF POKKAH BOENG DISEASE



FIG. 1B: CHLOROTIC PHASE II OF POKKAH BOENG



FIG. 1C: CHLOROTIC PHASE III TOP ROT OF POKKAH BOENG DISEASE

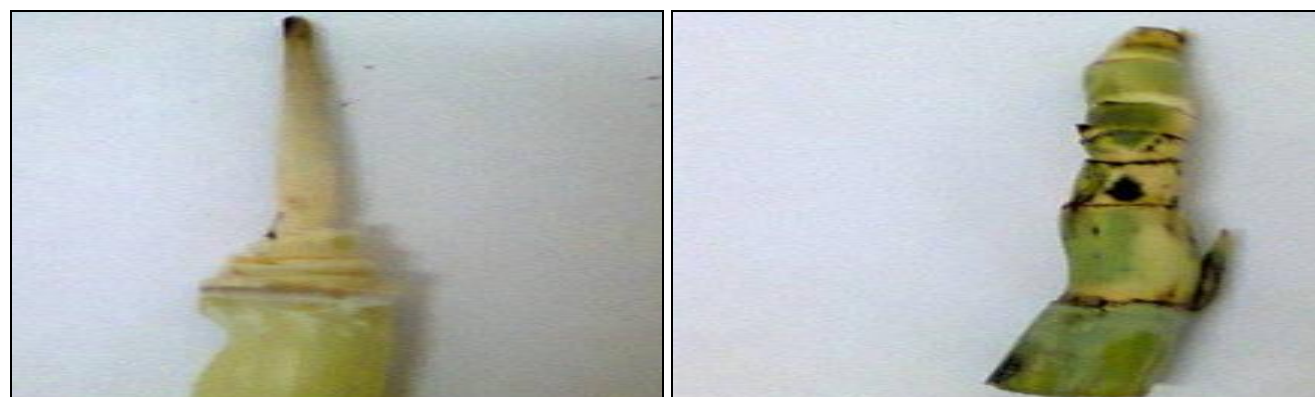


FIG. 1D: CHLOROTIC PHASE IV KNIFE CUT OF POKKAH BOENG DISEASE

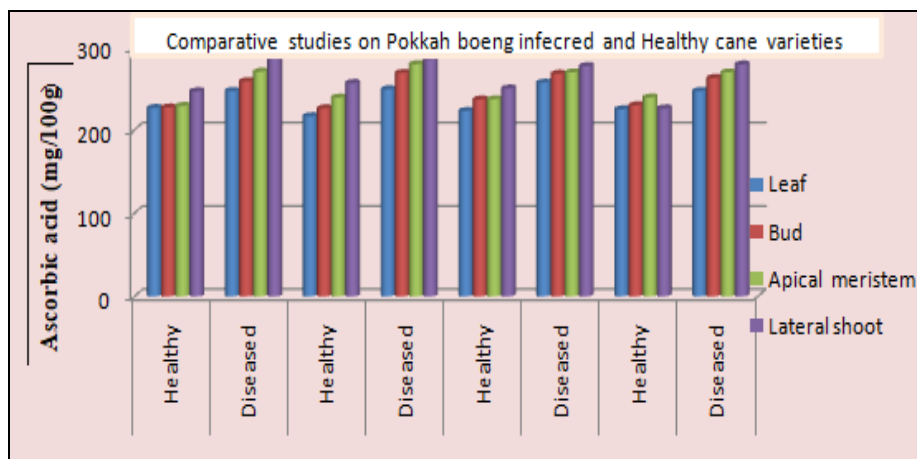


FIG. 2: CHANGES IN ASCORBIC ACID CONTENT IN POKKAH BOENG AFFECTED STALKS OF FOUR CANE VARIETIES

Isolation and Delineation of Microbial Strains from Rhizosphere Including Root Stubble from the Farm:

The soil used in the study was collected from the experimental farm of Genda Singh Sugarcane Breeding & Research Institute, Seorahi, Kushinagar. To assess the rhizospheric dynamics the soil samples were collected from a sugarcane field. Generally the native microbial consortium shows their resiliency to enhance the growth and development of the standing crop.

Microorganisms associated with rhizosphere are essential in nutrient transformation in a unique way and finally release for plant intake. The isolated microorganisms were screened *in-vitro* concerning their ability to assist Plant growth **Table 1**. The common nutrient agar medium was used to culture the microbial consortium. The variety of microbes including PGPR was dominant in the region with performance of different growth pattern.

Physical Characteristics	Texture-smooth; Color-Whitish cream; Elevation-Flat Rod shaped
Morphological Characteristics	
Citrate utilization	-
Nitrate reduction	+
Motility at 37 °C	+
Salt tolerance	+
Catalyst test	+
Gram stain	+

RESULTS AND DISCUSSION: The results indicate that there was an increase in the ascorbic acid content in leaf, bud, apical meristem and lateral shoots of *Pokkah boeng* affected stalks of both the test varieties *viz.* CoS 98259, CoS 8432, CoS 8436 and CoSe 01424 in comparison to control **Fig. 2**. However, the accumulation of ascorbic acid was maximum in tissues of apical meristem of affected stalks of CoS98259 (227.2mg/100g), CoS8432 (231.22mg/100g), CoS8436 (229.82mg/100g) and CoSe01424 (221.6 mg/100g or ml). The ascorbic acid content was also higher in the juice from diseased stalks in comparison to juice from control ones. Both increase and decrease in the ascorbic acid oxides in plant tissue have been reported following fungal infections. It is supposed that the augmentation in the ascorbic acid content in *Pokkah boeng* affected stalks reveals to be due to the production of ascorbic acid accelerating enzymes by the pathogen or by the interaction by parasite to the host. In this

it can be concluded that *Pokkah boeng* is now limiting the plant growth and playing a very important causing economic threats. The resiliency of the epidemiology of *Pokkah boeng* comes under minor concern but these days it will be major due to their rapid spread during the last few years. Nowadays, the frequency and severity of *Pokkah boeng* disease has been reported from major sugarcane growing states like Uttar Pradesh which seems to be alarming sign for future prospects.

Plant growth-promoting rhizobacteria (PGPRs) directly reveal the continuing need for new bioinoculants to be used on crops. Free-living bacteria that actively colonize plant roots and provide positive effects on plant development are called plant-growth promoting. These bacteria can promote plant growth in variety of ways including the use of their own metabolism to solubilize phosphates, produce hormone, fix nitrogen and natural resource management. PGPR also increase plant absorption of water and nutrients, improving root development and increasing plant enzymatic activity; moreover, PGPR promote other microorganisms as part of a synergistic effect to improve their effects on plants, promoting plant growth leading to increase productivity and sustainability.

One gram of rhizospheric soil was homogenized in 20 mL test tube containing 9 mL saline (0.85% NaCl) separately. The suspension was vortexed and dilutions were prepared up to 10^{-7} . Each dilution (0.1 mL) was spread on plates containing medium finally incubated at 30 ± 2 °C for 48 h. The bacterial colonies were distinguished on the basis of morphology and different biochemical tests **Table 1**. Biochemical metabolites are very beneficial in plant growth promotion and enhancing its activity. These results indicate that bacteria with the potential for use as future inoculants. PGPR may be utilized to augment plant growth and suppress plant pathogens.

CONCLUSION: As the presence of ascorbic acid in sugarcane plays a key role as a major non-enzyme antioxidant and mediates certain oxidative stresses caused by biotic and abiotic stresses. The disease free plants having the optimal conditions explore well in field with quality and quantity during production. The different kind of

biomolecules in sugarcane show diverse role with interaction of microbial consortium in soil and finally the production depends upon thresh hold limit of various agro climatic factors. The bioactive composition of ascorbic acid in sugarcane have shown endurance ameliorating the holistic knowledge to conduct research covering diverse arena for future prospects.

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