

ACC DEAMINASE POSITIVE BACTERIA IN STRESSED AGRICULTURAL ENVIRONMENTS

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by

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Dedicated

To

My family

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Agriculture, the backbone of uprising Indian economy, is under tremendous pressure to meet the requirements of ever-increasing population. Moreover, there is a constant threat to environmental health with the use of chemical amendments. Hence there is an eminent need for an eco-friendly, long term and sustainable boost in agricultural productivity in the presence of emanating climatic adversities and environmental deterioration. While microbes have been in application in agriculture since decades, they are still not considered an economical alternative because of their poor performance and survivability in field due to competition by resident microflora. ***The objective of the present study was to come up with potent bacterial strains that could serve as plant probiotic and stress buster for pigeonpea under adverse conditions. Hence the focus was on selection of indigenous ACC deaminase producing strain with multiple plant growth promoting (PGP) properties and better survivability in vivo. Besides the non-target impact of such an application was to be established to ascertain ecological health.*** ACC deaminase protects the plant from abiotic and biotic stresses by specifically reducing the deleterious stress ethylene levels. Enumeration of ACC deaminase bacteria was done from rhizosphere of pigeonpea from a control experimental field (without salinity stress), as well as from an experiment designed to simulate salinity and drought stress conditions. Isolates were shortlisted on the basis of various PGP properties such as indole acetic acid production, siderophore production, nitrogen fixation, HCN production and salt tolerance. The isolates were then assessed for plant growth promotion and mitigation of salinity stress under controlled conditions, followed by validation in field. Further, to

understand the impact such an application has on resident microbiome, molecular microbiology techniques of denaturing gradient gel electrophoresis and Illumina sequencing were applied. These bioinoculants not only proved to be efficient in mitigating the stress and enhancing plant growth, but also enriched the soil by its non-target impact of shaping the microbiome towards a more beneficial bacterial community. These isolates hold potential for dual benefit, i.e. plant growth promotion and plant protection, under stressed agricultural environments. *With a comprehensive polyphasic study from lab to field the potency of ACC deaminase producers has been established, which opens avenues for their widespread application to a range of crops and other types of stresses.*

कृषि, बढ़ती भारतीय अर्थव्यवस्था की रीढ़ है, और बढ़ती जनसंख्या की आवश्यकताओं को पूरा करने के लिए बहुत दबाव में है। इसके अलावा रासायनिक संशोधनों के उपयोग से पर्यावरणीय स्वास्थ्य के लिए लगातार खतरा है। इसलिए कृषि उत्पादन को इको-फ्रेंडली, दीर्घावधि और स्थायी रूप से बढ़ावा देने की आवश्यकता है। वह भी बढ़ती जलवायु विकृतियों और पर्यावरणीय गिरावट की उपस्थिति में। सूक्ष्मजीव कृषि में दशकों से उपयोग कर रहे हैं, लेकिन उन्हें अभी भी एक किफायती विकल्प नहीं माना जाता है जिस का कारण है उनकी फील्ड में खराब प्रदर्शन और निवासी माइक्रोफ्लोरा द्वारा प्रतिस्पर्धा के कारण उनका उत्तरजीविता खराब होना। वर्तमान अध्ययन का उद्देश्य है शक्तिशाली जीवाणु उपभेदों को प्राप्त करना जो की तनाव की स्थिति में कजानस कजान के लिए पौधे की वृद्धि और तनाव नाशक के रूप में कार्य कर सके। इसलिए प्राथमिकता यह थी की निवासी एसीसी डेमिनमिनस जीवाणु का चयन किया जाया जिसमें अनेक पौधों की वृद्धि को बढ़ावा देने (पी.जी.पी) के गुणों और फील्ड में बेहतर उत्तरजीविता हो। इस अध्ययन का उद्देश्य यह भी था की इस तरह के आवेदन के पारिस्थितिक स्वास्थ्य का पता लगाने के लिए गैर-लक्ष्य प्रभाव स्थापित किया जाना था। ए.सी.सी डेमिनमिनस विशेष रूप से एथिलीन के हानिकारक स्तर को कम करके पौधे को सभी अजैविक और जैविक तनावों से बचाता है। ए.सी.सी डेमिनमिनस जीवाणु की गणना कजानस कजान के रहिजोस्फीयर से की गयी थी जिसमे एक नियंत्रण प्रायोगिक क्षेत्र (लवणता तनाव के बिना) के साथ ही लवणता और सूखे तनाव की स्थिति का अनुकरण करने के लिए एक प्रयोग शामिल थे। उपभेदों के विभिन्न पी.जी.पी गुणों जैसे इंडोल एसिटिक एसिड उत्पादन, साइडरोफोर उत्पादन, नाइट्रोजन नियतन, एच.सी.एन उत्पादन और नमक सहिष्णुता के आधार पर शॉर्टलिस्ट

किया गया था। पौधों की वृद्धि को नियंत्रित करने और नियंत्रित परिस्थितियों में लवणता के तनाव को कम करने की आइसोलेट्स की क्षमता का आकलन भी किया गया था। इसके बाद क्षेत्र में सत्यापन की गयी थी। इसके अलावा, इस तरह के एक अनुप्रयोग के प्रभाव को समझने के लिए निवासी माइक्रोबायोम, आणविक माइक्रोबायोलॉजी तकनीकों में डीएन्ट्यूरिंग ग्रेडिएंट जेल वैद्युतकणसंचलन और इलुमिना अनुक्रमण किए गए थे। ये बायोइनोकुलेंट्स न केवल तनाव को कम करने और पौधे के विकास को बढ़ाने में कुशल साबित हुए, बल्कि माइक्रोफ्लोरा में अधिक लाभकारी जीवाणु समुदाय की ओर आकार देने के अपने गैर-लक्ष्य प्रभाव से मिट्टी को समृद्ध किया। इन आइसोलेट्स के दोहरे लाभ हैं, पौधों के विकास में बढ़ोतरी और पौधों की सभी अजैविक और जैविक तनावों से सुरक्षा। प्रयोगशाला से लेकर ए.सी.सी डेमिनमिनस उत्पादकों की क्षमता को व्यापक पॉलिफ़ैसिक अध्ययन के साथ स्थापित किया गया है, जो फसलों और अन्य प्रकार के तनावों के लिए उनके व्यापक अनुप्रयोग के लिए मार्ग खोलता है।

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ABBREVIATIONS

Elements and Chemicals

Ca²⁺: Calcium ion

Mg⁺: Magnesium ion

K⁺: Potassium ion

Na⁺: Sodium ion

(NH₄)₂SO₄: Ammonium sulphate

H₃BO₃: Boric acid

CTAB: Cetyl trimethyl ammonium bromide

DEPC: Diethyl pyrocarbonate

EDTA: Ethylenediaminetetraacetic acid

H₂O₂: Hydrogen peroxide

MoO₃: Molybdenum trioxide

NBT: Nitro tetrazolium

NaCl: Sodium chloride

NaOH: Sodium hydroxide

Media

D&F: Dworkin & Foster

LB: Luria Bertani

NA: Nutrient agar

NBRIP: National botanical research institute phosphate solubilizing

PAF: Pseudomonas agar F

TSB: Tryptic soya broth

Important terminology

ACC: 1-aminocyclopropane-1-carboxylic acid

acdR: Gene encoding leucine responsive regulatory protein (LRP; negative regulator of *acdS*)

acdS: Gene encoding ACC deaminase enzyme

ACO: ACC oxidase
ACS: ACC synthase enzyme
bp: Base pairs
CAS: Chroma azurole S
CBZ: Carbamazepine
cDNA: complementary DNA
CFU: Colony forming units
CID: Chemically induced drought
CLIN: Clindamycin
DAS: Days after sowing
DGGE: Denaturing gradient gel electrophoresis
EC: Electrical conductivity
FW: Fresh weight
g: Gram
G-ACC: γ glutamyl-ACC
Ha: Hectare
HS: High salinity
IAA: Indole acetic acid
JA-ACC: Jasmonoyl-ACC
kDA: kilodalton
kg: Kilogram
M-ACC: Malonyl-ACC
MB: Mega-byte
Mha: Million hectare
min: Minutes
s: Seconds
MPa: Megapascal
MS: Medium salinity

MSI: Membrane stability index

NID: Naturally induced drought

OTU: Operational taxonomical unit

PGP: Plant growth promoting

PGPR: Plant growth promoting rhizobacteria

POD: Peroxidase

PS: Phosphate solubilization

rpm: Rotations per minute

RT: Reverse transcriptase

RWC: Relative water content

SAM: S-adenosyl-L-methionine

SOD: Superoxide dismutase

TNA: Total nucleic acid

ANOVA: analysis of variance

DMRT: Duncan's multiple range test

FLASH: Fast length adjustment of short reads

QIIME: Quantitative insight into microbial ecology

UPGMA: Unweighted pair group method with arithmetic mean