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Overexpressing Carbonic Anhydrase Transgenic Rice Plants Maintain the Regular Soil Functions and Microbial Activities of Soil

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ABSTRACT

Background: Genetically modified (GM) crops are focused on establishing desirable traits to support the food demand of increasing populations. The effect of transgenic plants on soil diversity has been highlighted by a lot of researchers. Some of them showed the negative effect of transgenic crop on soil microbes and the food chain. So there is an urgent need to develop transgenic plants with no harmful effects on environmental health.

Methods: We have examined the effects salt tolerant carbonic anhydrase overexpressing transgenic rice plants on the physiology of rhizospheric microbes and their enzymatic activities. In the present study, we have used pot experiments in the greenhouse of Centurion University of Technology and Management, Bhubaneswar, Odisha, India.

Result: No significant variation in the soil properties *viz*., pH, Eh, organic C, P, K, N, Ca, Mg, S, Na and Fe^{+2} were observed. The population of bacteria, fungi and nematodes were remained same in the soil. The enzymatic activities like soil dehydrogenase, nitrate reductase, urease and alkaline phosphatase were not significantly affected. Further, plant growth promotion (PGP) functions such as HCN, siderophore, salicylic acid, GA, IAA, zeatin, were, not influenced considerably. The present study ecologically pertinent of salt tolerant CA transgenic rice to usual functions of the rhizospheric organisms.

Key words: Carbonic anhydrase, Dehydrogenase, GM crop, Salinity, Soil microorganisms, Transgenic rice.

INTRODUCTION

The most challenging factors of food security are due to increasing population, insufficient resources and rising needs. Climate change, diseases and reduced soil fertility rates caused by pests and pathogens can lead to unpredictable weather patterns, lower crop yields and economic losses (Naheed *et al*., 2023). GM crop cultivation has helped with feeding the world′s growing population. In 2017, 24 countries cultivated 1.898 billion hectares of transgenic plants (He *et al*., 2019). Transgenic crops have been found to increase farmer revenues by 68%, reduce pesticide costs by 39% and raise crop yields by 22% (Caradus, 2022). These benefits shows the potential of transgenic crops to contribute to a more sustainable and efficient agricultural industry (Carzoli *et al.,* 2018). For this reason, transgenic cultivars have higher seed costs so farmers get high profit and Non-financial benefits like increasing organizational flexibility, saving time and convenience of use (Baghbani *et al*., 2021). GM crops has more benefits and it is proved over the past 20 years yet consumers are not seeing any results (Brookes *et al*., 2014). Consumers are worried about allergies, unexpected changes in nutritional quality, the effects of ingesting ″foreign″ DNA and potential health hazards connected to genetically modified food. Eating conventionally grown plant is also associated with risks to the environment and biosafety, particularly when the mode of gene transfer is unclear (Herman *et al*., 2013). Transgenic plants with foreign genes may have harmful ecological effects, such as improved survival rates and competitive capacities among the same or various kinds of organisms. This may lead to spread, transfer of genes to natural species, enhancing the resistance mechanisms of target pests and direct or indirect effects on non-target animals and ecosystems (Machado *et al.,* 2020. Many researches were carried out and it was found GM crops have actually negative effect on soil fertility and health (Lebedev *et al*., 2022). The soil ecosystem is vital for ensuring the developmental progress of the plant.The plants absorb nutrients with water through the root system and the roots of plants adapt and sense to variation in other stresses and soil nutrient levels by regulating their physiological activity (Sun *et al*., 2020). The relationship between bacteria and plants in the root rhizosphere is very much complex. Microorganisms in the

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root environment are proactively maintained by plants through the transfer of organic compounds from the leaves to the roots and rhizosphere (Dhali *et al.,* 2019; Das *et al.,* 2021; Sahoo *et al*., 2023). We used transgenic rice seedlings that were previously produced in our lab and that overexpress CA and confer salt tolerance. We examined the impact of transgenic rice plant on microbial populations in the rhizosphere to comprehend the possible effects on the soil rhizosphere.

MATERIALS AND METHODS

Experimental site, soil sampling and salt stress treatment

The greenhouse pot experiments were carried out in Centurion University of Technology and Management (20°17′50.604″N; 85°50′26.232″) E), Bhubaneswar, Odisha, India. Each pot was filled with soils (separately with three replications). Approximately Twenty-one-day-old T1 seedlings from the CA over expression lines were cultivated along with wild type (WT) of the same variety (IR64) with a constant supply of 200 mM NaCl. All of the pots were immersed in 200 mM NaCl using the square metal container.

Chemical, physical and enzymatic characteristics of soil in the rhizosphere

Estimates for the following were made right away in the research laboratory using the established procedures: available nitrogen (AN) (kg ha), available sodium (ANa) (kg ha^{-1}),organic carbon (OC) (%), calcium (ACa) (kg ha^{-1}), iron (Fe^{2+}), phosphorus (AP) (kg ha^{-1}), potassium (AK) (kg ha^{-1}) ,magnesium (AMg) (kg ha^{-1}) and sulfur (AS) (kg ha^{-1}) ((Jackson, 1975). The soil's enzymatic activities, such as those of urease (Sahoo and Tuteja, 2013), nitrate reductase (Abdellmagid and Tabatabai, 1987), dehydrogenase (Min *et al.,* 2001) and alkaline phosphatase (Sahoo *et al*., 2015) were evaluated.

Plant growth promoting (PGP) activities of the selected isolates

All plant growth promoting traits *viz*; Siderophore, Salicylic acid, IAA, GA3, Zeatin, Abscisic acid, Zeatin, P release, ACC deaminase, NH_3, iron tolerance were carried out by Sahoo and Tuteja (2013).

Statistical data analysis

Three distinct replicates' average mean value was utilized to compute the standard error (SE).Graphs were plotted with GraphPad Prism 8.2.1 (GraphPad Software, Inc., San Diego, CA).

RESULTS AND DISCUSSION

Physical and chemical characteristics of experimental soil

The current investigation was focused on the CA OE and WT plants' physical and biochemical properties in the soil's rhizospheric zone. The collected top soil included a variety of compositions, including alluvial soil and sandy loam-type soil with 8.3% sand, 4.2% silt, 17.4% clay and 2.71 g cm^{-3} particle density. There were variations in the soil's pH between 6.49 and 7.12, electrical conductivity (EC) between 0.59 dS m^{-1} and 0.66%, organic carbon, phosphorus, potassium, calcium and accessible nitrogen, magnesium and sulphur with phosphorus being 28 kg ha^{-1}, potassium being 104 kg ha^{-1} and sulfur being 19 kg ha^{-1} (Table 1). Several findings have shown that growing transgenic crops had no impact on the physio-chemical characteristics of the soil, enzyme activity, or soil biodiversity (Wu *et al*., 2021, Chen *et al*., 2022, Sahoo *et al*., 2015). It is

Table 1: Physiochemical properties of the soil.

Properties				
Soil Texture		Alluvial soil to sandy loam		
Plant characteristics	*CA* Control	*CA* (Salt)	Wild type	Wild type (Salt)
Sand (%)	86.5	86.5	86.5	86.5
Slit (%)	9.8	9.8	9.8	9.8
Clay (%)	3.7	3.7	3.7	3.7
Particle density (g cm^{-3})	3.61	3.61	3.61	3.61
Bulk density (g cm^{-3})	3.09	3.09	3.09	3.09
Organic carbon (%)	0.87	0.87	0.87	0.87
pH	6.32	7.21	6.32	7.21
Electrical conductivity (dS m^{-1})	0.42	0.49	0.42	0.49
Available Calcium (kg ha^{-1})	98.5	98.5	98.5	98.5
Available magnesium (kg ha^{-1})	122.6	122.6	122.6	122.6
Available nitrogen (kg ha^{-1})	111.2	111.2	111.2	111.2
Available phosphorus (kg ha^{-1})	27.5	27.5	27.5	27.5
Available potassium (kg ha^{-1})	108.1	108.1	108.1	108.1
Available sodium (kg ha^{-1})	17.2	23.8	17.2	23.8
Available sulfur (kg ha^{-1})	16.9	16.9	16.9	16.9

WT: Wild type rice; *CA*: transgenic rice overexpressing Carbonic anhydrase gene.

interesting to note that, in the current investigation no significant differences were observed in the enzyme activity of transgenic crop rhizospheric soil.

Rhizospheric soil microbial population profile

The impact of CA over expression line development on the soil microbiota of the collected soils was investigated, both with and without salt treatment (Fig 1A, 1B, 1C, 1D). In WT control pots, the population dynamics of rhizospheric bacteria were 61×10^5 cfu g^{-1}. Under control situations, it was found to be 62×10^5 cfu g^{-1} in the pots containing the CA over expression lines. As compared to their pre-salt treatment status, certain prominent soil nematodes exhibit a little drop in population following the salt treatment. The nematode population in each pot of soil under control (each pot contained around 8 kg soil) varied from 953 in the WT pot to 948 in the soil containing the CA over expression lines. Nematode populations per pot for plants under salt stress are 940 for WT plants and 941 for over expression lines, respectively. According to our research, the soil microbiome was not significantly affected by the transgenic CA rice plants. These findings were consistent with earlier research on herbicide-resistant transgenic rice (to protoporphyrin oxidase (PPO)-inhibiting) and transgenic rice MSRB2-Bar-8, where tests on soil microbial communities showed no negative effects (Sohn *et al*., 2016;

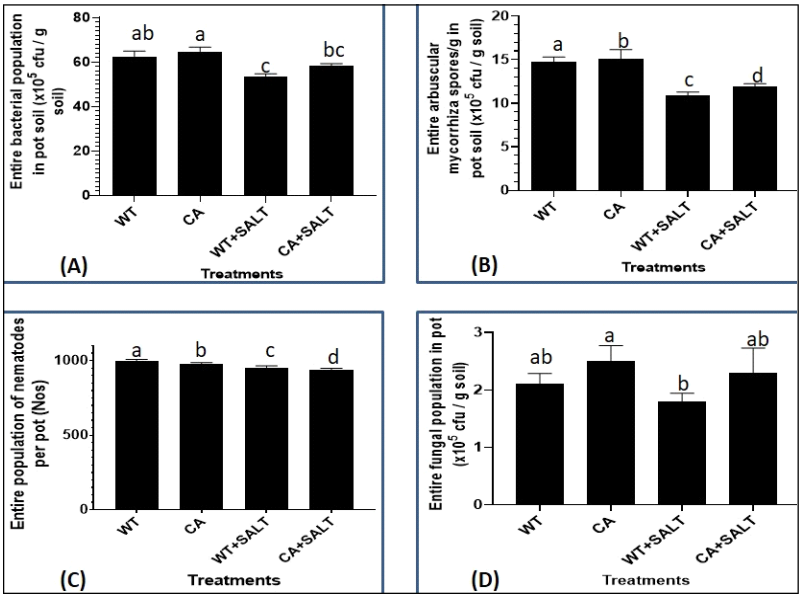


Fig 1: A) Entire bacteria population, B) Entire mycorrizal spore count, C) entire nematode population, D) entire fungal population.

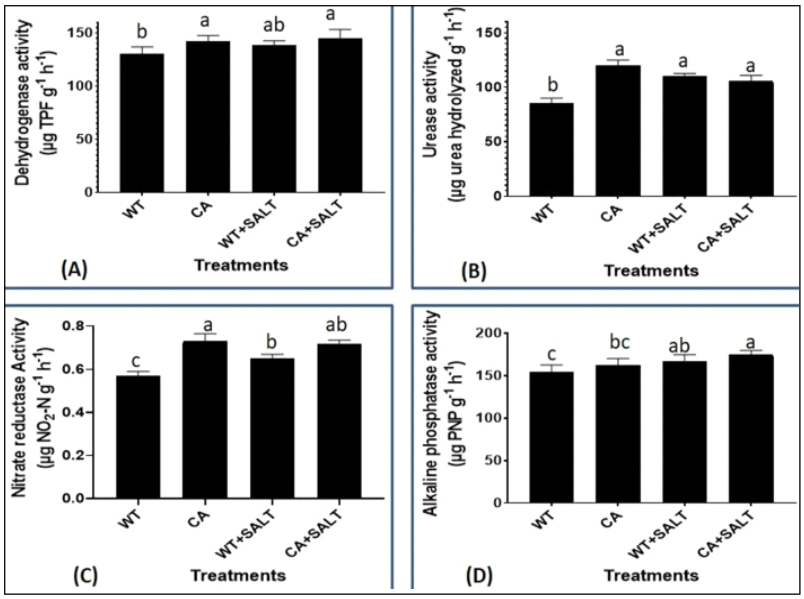


Fig 2: A) Dehydrogenase activity, B) urease activity, C) nitrate reductase activity, D) Alkaline phosphatase activity.

Table 2: PGP functions of five bacterial colonies in rhizospheric soil of WT and CA transgenic plant under salt stress (200 mM NaCl) and control conditions in collected soil.

Sample	Siderophore Isolate no.	HCN (µM/ml)	Salicylic acid (A625)	IAA (µg/ml)	GA_3 (µg/ml)	Zeatin (µg/ml)	ABA (µg/ml)	P release (ng/ml)	ACC (µg/g dr. wt)	NH3 deaminase (nM α- KBT/ mg/h)	Iron (mg/ 10 ml broth)	tolerance (mg/l)
WT	1	0.58	-	-	-	0.32	-	-	-	-	0.61	95
	2	-	-	0.12	6.82	-	-	-	-	-	1.21	80
	3	-	-	-	-	0.12	-	-	37.86	-	0.42	120
	4	0.4	-	-	-	-	0.02	-	-	-	0.58	120
	5	-	0.02	-	-	-	-	0.22	-	-	0.11	90
WT + salt	1	-	-	0.23	0.96	-	-	-	-	22.92	0.05	90
	2	-	-	-	2.52	-	-	-	39.56	-	0.34	140
	3	0.41	-	-	-	0.04	-	-	-	-	0.05	140
	4	-	-	0.18	-	-	-	0.26	-	-	0.31	120
	5	0.38	0.03	-	-	-	-	-	-	13.36	0.05	80
CA	1	-	-	-	-	0.06	-	-	-	-	0.86	95
	2	-	-	0.12	-	-	0.04	-	-	-	0.84	65
	3	-	-	-	4.72	-	-	-	-	8.68	0.16	80
	4	-	-	0.16	-	-	-	-	61.72	-	0.06	120
	5	0.44	-	-	-	-	-	0.38	-	-	0.25	160
CA + salt	1	-	0.02	-	-	-	0.06	-	-	-	0.76	110
	2	-	-	-	3.18	-	-	-	-	-	0.54	170
	3	-	-	-	2.85	-	-	-	-	18.42	0.40	120
	4	-	0.06		-	-	-	-	60.18	-	0.92	80
	5	-	-	0.16	-	-	-	-	12.06	-	0.05	160

Chen *et al*., 2022). This may be ascribed to that there may not be any toxic secretion in transgenic plant rhizosphere. An essential part of the ecology and health of a plant is the soil. The primary regulators of soil physio-chemical properties and fertility are the soil enzymes (Pahalvi *et al*., 2021). Based on microbial populations, enzyme activity may provide important information regarding changes in the soil. According to previous research, there has been a lack of variation in the makeup of the rhizosphere, while others have highlighted the detrimental effects of transgenic crops (Lebedev *et al*., 2022). Here marker free transgenic crops are of sustainable importance, as they must not secret undesirable compounds to the environment. However, the long-term planting of GM agricultural plants may or may not have an influence on the variety of the soil microbial community such as *Bacillus*, *Pseudomonas*, *Rhizobium etc.* (Lebedev *et al*., 2022, Meena *et al*., 2020). An earlier publication reported that SUV3 transgenic rice had no effect on the microbiota of the rhizospheric soil (Sahoo *et al*., 2015). This study supports the findings of beta-carotene transgenic rice, which did not affect bacterial colonies at different stages of growth or rhizosphere enzyme activity (Li *et al*., 2014). As in the current investigation, no adverse impact on soil microbiota was observed in both transgenic and control plants.

CA transgenic rice poses no significant change on enzymatic activities of rhizospheric soil

Soil dehydrogenase activity measured in soil samples taken from transgenic rice plants CA T1 and WT control plants was 151 μg TPF g^{-1} h^{-1} and 137 μg TPF g^{-1} h^{-1}, respectively. In WT plants, the urease activity increased to 105 μg g^{-1} h^{-1} under salt treatment, whereas in CA T1 transgenic rice lines, it reached to 116 μg g^{-1} h^{-1}.The WT control plants and CA T1 transgenic rice had soil nitrate reductase levels of 0.52 μg NO_2 g^{-1} h^{-1} and 0.71 μg NO_2 g^{-1} h^{-1}, respectively. The WT control plant and the CA T1 transgenic rice plant had soil alkaline phosphatase levels of 174.00 μg PNP g^{-1} h^{-1} and 175.00 μg PNP g^{-1}, respectively (Fig 2A, B, C and D). Previous study analysis demonstrated that nitrate reductase, phosphatase and urease enzyme activity decreased throughout the harvesting period (Ito *et al*., 2020). Soil enzymatic pattern have not shown significant impact due to transgenic plant. This may be attributed to the fact that transgenic plant root rhizosphere secretions were not having any toxic compounds to hamper the soil enzymes. Soil rhizospheric secretions must be studied to validate this statement. In contrast, there were little differences in the activity of these enzymes in the soil rhizospheric zone between the transgenic and wild-type plant lines in the current research. During the early vegetative and floral phases growth period (150 days), the rhizosphere was very active with urease, alkaline phosphatase and nitrate reductase, while the gall formation phase saw a significant enzyme response rate from dehydrogenase. The results of earlier research on SUV3 transgenic rice, BcWRKY1 transgenic lines of maize and BADH-overexpressed transgenic lines of maize are in line with this (Sahoo *et al*., 2015; Zeng *et al*., 2022). Research on Bt-cotton containing Cry1Ac or Cry1Ab/Ac also indicated that the diversity of the nematodes, *arbuscular mycorrhiza* and bacterial population were not significantly affected (Yang *et al*., 2014). Our results clearly show that, under both control and stress settings, the soil microbe population and its enzyme activity were not adversely affected by the growth of CA rice transgenic plants (Lebedev *et al.,* 2022).

Plant growth promotion (PGP) functions of the isolated rhizospheric soil bacteria

The rhizospheric soils of the WT and CA over expression lines were subjected to an analysis of their bacterial activities. These activities included siderophore production, phosphorus release, salicylic acid and hydrogen cyanide, cytokinin, abscisic acid, gibberellic acid, ammonia, 1-amino cyclopropane-1-carboxylic acid deaminase and iron tolerance. For both the WT and CA over expression lines, the PGP functions of all the split PGPRs were found to be similar under all tested circumstances, with no discernible variations (Table 2). The current study's PGP actions of the rhizospheric bacteria isolated from these experimental conditions showed no change in the physiological processes of microorganisms, as did the soil rhizospheric area of WT and transgenic plant lines overexpressing CA. The PGP functions experiment findings corroborate the idea that rice plants overexpressing salt-tolerant CA did not significantly harm the soil's microbial population or its activities (Sahoo and Tuteja, 2013).

CONCLUSION

Strong evidence of the non-toxic impact of transgenic crops on soil microbiota and enzymatic activities was presented by the current study. To better understand our transgenic lines' potential applications and user advantages, needs further work in terms of protein and organic acid analysis of soil rhizosphere which can provide a better understanding on the impact of transgenic crop on soil biological activity.

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Conflict of interest

The authors declare no conflict of interest.

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