Andhra Pradesh J Agril. Sci : 10(1): 54-58, 2024

ENHANCING NUTRIENT CONTENT OF CHICKPEA GRAINS THROUGH PLANT GROWTH-PROMOTING ACTINOBACTERIA

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Date of Receipt: 27-02-2024 Date of Acceptance: 25-03-2024

ABSTRACT

This study highlights the potential of 26 different strains of beneficial microorganisms, including 19 *Streptomyces* sp. (CAI-13, CAI-17, CAI-21, CAI-24, CAI-26, CAI-68, CAI-78, CAI-85, CAI93, CAI-121, CAI-127, CAI-140, CAI-155, KAI-26, KAI-27, KAI-32, KAI-90, KAI-180 and MMA-32) and 7 system of rice intensification (SRI) bacterial isolates (SRI-156, SRI-158, SRI-178, SRI-211, SRI-229, SRI-305, and SRI-360), in enhancing the nutrient content of chickpea grains. These beneficial microorganisms have been found to be effective in various crops such as chickpea, pigeonpea, sorghum, rice, pearl millet, tomato, and chili. The field experiment was conducted to evaluate the impact of these plant growth-promoting actinobacteria on seed nutrition in chickpea. The results showed that inoculating the seeds with these microorganisms significantly improved the nutritional values of the chickpea seeds compared to the un-inoculated control. All the *Streptomyces* sp. were found to enhance the crude protein (up to 12.4%), crude fiber (up to 46.3%), crude fat (up to 33.3%), and total ash (up to 25%) contents of the seeds over the uninoculated control seeds. The bacterial strains also improved the crude protein (up to 13.8%), crude fiber (up to 49.4%), crude fat (up to 19.3%), and total ash (up to 14.3%). Hence, it is concluded that beneficial microbes can serve as a complementary sustainable tool for the existing biofortification strategies.

KEYWORDS: Plant growth-promoting actinobacteria, System of rice intensification (SRI), *Streptomyces* sp. Chickpea, Biofortification Minerals.

INTRODUCTION

Malnutrition is a persisting global calamity that is prevalent mainly in Africa and South Asia. It exists in three aspects: undernutrition (stunting, wasting, and underweight), obesity, and malnutrition associated with micronutrient deficiency (hidden hunger). The World Health Organization (WHO) estimates over 2 billion people suffer from hidden hunger (Ritchie and Roser, 2017). At the same time, 150.8 million, 50.5 million, and 38.3 million children aged below 5 years are stunted, wasted, and overweight, respectively (Ritchie and Roser, 2017; Global Nutrition Report, 2018). South Asian women and school children are highly vulnerable to malnutrition. One-third of women of reproductive age are anemic and show higher susceptibility to obesity than men (Global Nutrition Report, 2018). Plant breeding and agronomical practices introduced in the 1960s during the green revolution primarily combatted global hunger, especially through large-scale cereal production, providing the necessary calories or proteins to these vulnerable populations (Thavarajah *et al.,* 2014; Roorkiwal *et al.,* 2021). Therefore, biofortification through PGPR (plant growth promoting rhizobacteria) is a sustainable alternative to combat this hidden hunger or micronutrient malnutrition. The utilization of PGPR for biofortification not just encourages us to manage the issue of malnutrition among the population, in addition, improves crop yield, soil fertility, and biodiversity. Biofortification through rhizobacteria has gained popularity to improve Zn and other micronutrient contents in grain crops. For instance, Gopalakrishnan et al. 2016c, Satya et al. 2016, Srinivas *et al.,* 2021 revealed the species from the genera *Streptomyces*, *Pseudomonas*, *Brevibacterium*, *Bacillus*, *Enterobacter*, and *Acinetobacter* as potential candidates for biofortification and biocontrol in plants. These microorganisms also can incorporate micronutrients inside eatable plant tissues through solubilization of their indigenous insoluble sources present in the soil.

Actinomycetes, particularly those belonging to the genus *Streptomyces*, can induce antioxidant enzymes in chickpea leaves, potentially leading to improved nutrient content in the grains. In recent years, researchers have explored the potential of actinomycetes to enhance the nutritional value of various crops, including chickpeas. Therefore, in the present study 26 bacterial strains including actinomycetes which are previously reported as plant growth promoting bacteria and as natural pest control agents from ICRISAT were tested for their nutritional improvement in chickpea grains by seed inoculation in a field study.

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MATERIALS AND METHODS

Bacterial isolates

This study investigates 26 different strains of PGP (plant growth-promoting) bacteria, found in herbal vermicompost and SRI (System of Rice Intensification) rice rhizosphere soils. Among these strains, 19 are *Streptomyces* species and 7 are SRI-bacterial isolates. These bacteria have been reported as PGP and biocontrol agents in various crops like chickpea, pigeonpea, sorghum, rice, pearl millet, tomato, and chilli (Gopalakrishnan *et al.,* 2011a; 2011b; 2011c; 2012a; 2012b; 2013a; 2013b; 2014; 2015a, 2015b; 2015 c; 2016a, 2016b, 2016c, 2016d; 2020a; 2021; Sathya et al. 2016; Sravani *et al.,* 2021, Srinivas *et al.,* 2020; 2023; Sambangi *et al.,* 2022). The genome sequences of 16 *Streptomyces* strains, which demonstrate potential for plant growth promotion (PGP) activities in numerous crops, have been successfully decoded. (Gopalakrishnan *et al.,* 2020b).

Effect of selected bacterial strains on nutrients concentration in harvested grains of chickpea under field conditions

The field experiment was carried during the post-rainy cropping season at ICRISAT (17°30' N; 78°16' E; altitude 549 m), Patancheru, India. The experimental site featured Vertisol soil, which is rich in clay content (52%), silt (21%), and sand (26%). The soil had an alkaline pH of 7.7-8.5 and an organic carbon content of 0.4-0.6%. The soil depth was at least 1.2 meters, and it could retain around 200 mm of plant-available water in a 120-cm soil profile. The top 15 cm of the rhizosphere soil contained 24.7 mg kg^{-1} of available nitrogen, 8.6 mg kg^{-1} of available phosphorus, and 298 mg kg^{-1} of available potassium. The field was kept fallow except for the post-rainy season crop. The land was prepared into broad beds and furrows, with beds measuring 1.2 meters wide and flanked by 0.3-meter furrows in both seasons. Fertilizers, including 18 kg N ha^{-1} and 20 kg P ha^{-1} as di-ammonium phosphate, were applied and incorporated into the soil. The experiment was conducted using a randomized complete block design (RCBD) with three replicates and plot sizes of 4 meters by 3 ridges (rows).

The 26 selected bacterial strains were cultured individually on Strach casein broth for *Streptomyces* sp., and Luria Bertani broth of SRI- bacterial strains. The chickpea seeds (ICCV 2) were treated with bacterial strains at a concentration of 10^8 CFU/ml for 40 minutes, then sown immediately by hand planting. The plants were sown in rows 30 cm apart with a depth of 5 cm, aiming for at least 26 plants per square meter. Throughout the growth period, until the flowering stage, the plants were inoculated with the respective bacterial strains every 15 days by applying them to the soil near the plants. In control plot were maintained without bacterial treatment. During the cultivation, no pesticides were used as no significant diseases or pests were observed. The crop was manually harvested. After crop maturity seeds were collected treatment wise, dried, grounded and then flour was stored in paper bags. The samples were properly labelled and sent to TNAU (Tamil Nadu Agricultural University) for analyzing crude protein (Kjeldahl method), crude fat (Soxhlet extraction method), crude fiber (AOAC method, 2005) and total ash.

RESULTS AND DISCUSSION

Among the 26 different strains of bacteria, all the *Streptomyces* sp. enhanced the crude protein (4% to 12.4%), crude fiber (4.4% to 46.3%), crude fat (2.7% to 33.3%), and total ash (14.3% to 25%) of chickpea seeds, same as all SRI-bacterial isolates have significantly improved the crude protein (0.3% to 13.8%), crude fiber (28.3% to 49.4%), crude fat (0.7% to 19.3%), and total ash (up to 14.3%) over the seeds of control plants (Table 1). The *Streptomyces* sp. and bacteria sp. used in this experiment were earlier reported to enhance the plant growth promotion in chickpea crop (Gopalakrishnan *et al.,* 2011a; 2011b; 2011c; 2012a; 2012b; 2013a; 2013b; 2014; 2015a, 2015b; 2015 c; 2016a, 2016b, 2016c, 2016d; 2020a; 2021; Sathya et al. 2016; Sravani *et al.,* 2021, Srinivas *et al.,* 2020; 2023; Sambangi *et al.,* 2022).

The results of present investigation were similar to the results of Gopalakrishnan *et al.* (2022) in which consortium of *Streptomyces* sp. have significantly improved the crude protein, crude fiber, crude fat and total ash content of chickpea grains. Also, the results are parallel with Srinivas *et al.,* 2021 wherein the *Streptomyces* strains have significantly increased the grain nutrient contents of pearl millet hybrids and showed a PGP based sustainable biofortification. The results are in line with Sathya *et al.,* 2016, where they evaluated the plant growth promoting actinobacterial cultures increased the seed mineral density of chickpea significantly. Gopalakrishnan *et al.* (2016) assessed bacterial cultures for their plant growth promotion and biofortification where they significantly improved the mineral content of grains in chickpea and pigeon pea crops. Therefore, it suggests that microbial based biofortification of seeds is a sustainable tool for existing biofortification strategies.

In this study, we examined the potential of 26 different strains of microorganisms, including 19 *Streptomyces* sp. and 7 SRI-bacterial isolates, to

Table 1. Effect of nutrient content of chickpea grains through plant growth promoting bacteria

Isolate	Crude protein %	Crude fibre %	Crude fat %	Total ash %
CAI-13	21.2***	2.3	7.30	3.0
CAI-17	20.8***	3.3**	10.15***	3.0
CAI-21	19.2	2.8	7.60	3.5***
CAI-24	22.2***	3.0	7.40	3.0
CAI-26	19.0	2.8	8.30	3.5***
CAI-68	21.5***	3.3**	10.65***	3.0
CAI-78	19.2	3.0	7.00	4.0***
CAI-85	20.3	3.8**	8.65***	3.0
CAI-93	23.8***	3.8**	6.40	4.0***
CAI-121	19.5	4.0**	9.40***	3.0
CAI-127	20.6***	3.0	8.45***	3.0
CAI-140	22.1***	3.3**	7.95	3.0
CAI-155	20.1***	2.8	6.80	3.0
KAI-26	21.4***	2.8	6.95	4.0***
KAI-27	22.1***	3.0	7.65	3.0
KAI-32	20.5	3.3**	7.15	4.0***
KAI-90	20.2	3.8**	7.70	3.0
KAI-180	21.9***	2.8	8.95***	3.0
MMA-32	21.2***	3.0	8.40***	3.5***
SRI-156	19.5	3.0	8.80***	3.0
SRI-158	21.9***	3.3**	8.40***	3.0
SRI-178	21.8***	3.8**	7.45	3.0
SRI-211	22.5***	4.3**	9.45***	3.0
SRI-229	21.0***	3.3**	8.80***	3.0
SRI-305	20.8***	3.5**	7.65	3.5***
SRI-360	20.5	3.5**	7.15	3.0
Control	19.4	2.2	7.10	3.0
Mean	20.9	3.2	8.1	3.2
LSD (5%)	1.19	0.91	1.32	0.53
CV%	3	14	8	8
CAI-13	21.2***	2.3	7.30	3.0
CAI-17	20.8***	3.3**	10.15***	3.0

LSD = Least significant differences; CV = Coefficients of variation;
** = Statistically significant at 0.01, *** = Statistically significant at 0.001

enhance the nutrient content of chickpea grains. The field experiment was conducted during the post-rainy cropping season at ICRISAT, Patancheru, India, to evaluate the impact of these plant growth-promoting actinobacteria on seed nutrition in chickpea. The results showed that inoculating the seeds with these microorganisms significantly improved the nutritional values of the chickpea seeds compared to the un-inoculated control. The *Streptomyces* sp. and bacterial isolates improved various nutritional parameters, such as crude protein, crude fiber, crude fat, and total ash content in chickpea grains. These findings suggest that microbial inoculum might be a complementary sustainable method for current nutritional approaches, potentially decreasing the utilization of chemical fertilizers in agricultural fields. The use of microbial-based biofortification of seeds can be considered a sustainable tool for existing biofortification strategies, contributing to combating malnutrition and improving crop yield, soil fertility, and biodiversity.

ACKNOWLEDGMENTS

This work has been undertaken as part of the CGIAR Research Program on Grain Legumes Dry Land Cereals.

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