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Enhancing Cowpea (*Vigna unguiculata*) Yield and Exploring Cross-inoculation Potential of Cowpea Native Rhizobia on *Phaseolus vulgaris* in Eastern Kenya

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ABSTRACT

Background: The cultivation of cowpea in Sub-Saharan Africa plays a crucial role in enhancing food security for the region's growing population, while also enriching the soil with vital nutrients such as nitrogen (N) through biological N fixation. However, current cowpea production falls far below its estimated potential. Additionally, a large percentage of cowpea is grown by smallholder farmers who face challenges such as poor soil fertility, low N levels and low yields. Therefore, this study aimed to investigate the effect of native rhizobia inoculation on field-grown cowpea and its cross-inoculation potential on common bean (*Phaseolus vulgaris*).

Methods: A field experiment was conducted based on randomized complete block design (RCBD) with three cowpea varieties and four treatments: indigenous rhizobia, comsmercial rhizobia, a consortium of both and an uninoculated control. Native rhizobia were isolate from root nodules in control plots, characterized morphologically and biochemically and identified *via* Sanger sequencing. These isolates were then cross- inoculated onto two common bean varieties in a greenhouse RCBD.

Result: Isolated rhizobia differed genetically across regions and included various *Rhizobium* species. Cross-inoculation significantly increased root dry weight, shoot dry weight, nodule number and nodule dry weight in common bean ($p<0.05$). Native rhizobia showed a strong potential as sustainable biofertilizers for improving legume productivity across varying climatic conditions.

Key words: Biofertilizer, Common bean (*Phaseolus vulgaris*), Cowpea (*Vigna unguiculata*), Cross-inoculation, Native rhizobia.

INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp.] is a vital legume in tropical and subtropical regions, especially in Sub-Saharan Africa. It provides affordable dietary protein, complements cereal-based diets and its pods and leaves are also consumed as vegetables (Oliveira *et al*., 2017; Ayalew *et al.,* 2021). Beyond nutritional benefits, cowpea improves soil fertility through biological nitrogen (N) fixation (BNF) and serves as feed and forage (Mulugeta *et al*., 2016; Vanlauwe *et al*., 2019; Sharma *et al*., 2025). Africa produces about two-thirds of global cowpea, worth over $1 billion annually (Ayalew *et al*., 2021). In Kenya, it is the third most widely grown legume, mainly in Eastern and Coastal Regions, contributing about 16% of total legume production (Ndungu *et al*., 2018; Kuruma *et al*., 2019). Despite its importance, productivity remains low because smallholder farmers contend with poor soil fertility, limited access to inputs and erratic rainfall (Owade *et al*., 2020; Muindi *et al*., 2017).

Cowpea is known for its promiscuous association with root-nodule colonizing bacteria, collectively known as rhizobia, forming symbioses with various species within the Alphaproteobacteria and Betaproteobacteria phyla (Andrews *et al*., 2017). Such symbioses enhance N fixation and reduce dependence on synthetic N fertilizers (Ndungu *et al*., 2018; Samudin and Kuswantoro, 2018). Rhizobia inoculation is a cost-effective and sustainable alternative to fertilizers, which enhances soil fertility, improves plant growth, yield and drought tolerance, particularly in arid regions (Sofi *et al*., 2018). However, commercial inoculants often perform poorly than native rhizobia which are better adapted to local soils (Mendoza-Suárez *et al*., 2021; Rathi *et al*., 2018), underscoring the importance of indigenous strains. Therefore, cross-inoculation, using native rhizobia from one legume host to another, could extend these benefits to crops like common bean (*Phaseolus vulgaris*) (Paudyal *et al*., 2021; Favero *et al*., 2022). While the rhizobia benefits are well established, effectiveness of cowpea-associated native rhizobia in nodulating common bean growth remains largely unexplored. Elucidating this potential is key to developing affordable, ecofriendly bioinoculants for smallholder farming systems.

This study aimed to bridge these knowledge gaps by investigating the impact of native rhizobia isolates on cowpea performance in Eastern Kenyan fields and exploring their cross-inoculation potential with common

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beans under controlled greenhouse conditions. We hypothesized that native cowpea-nodulating rhizobia from Eastern Kenya would significantly improve cowpea growth and yield and that these isolates could also enhance common bean yield upon cross-inoculation. Furthermore, we anticipated that these native isolates would effectively nodulate and promote growth of common bean varieties, suggesting their broader agricultural utility. To achieve these goals, this study focused on three main objectives: (i) to evaluate effect of native rhizobia inoculation on the growth and yield of three cowpea varieties in Eastern Kenyan fields; (ii) to characterize genetic, biochemical and morphological diversity of the native cowpea-nodulating rhizobia; and (iii) to determine cross-inoculation potential of these isolates on common beans (*Phaseolus vulgaris*) under controlled greenhouse conditions.

MATERIALS AND METHODS

Description of study sites

The study was conducted in three counties in Eastern Kenya: Embu, Tharaka Nithi and Kitui. These regions represent diverse agro-ecological zones, providing a robust setting for evaluating cowpea performance and rhizobia diversity (Table 1).

Land preparation

The land was prepared prior to onset of short rains in September 2021. Vegetation on the selected farms was cleared, followed by plowing and leveling. The plowed land was divided into smaller lots measuring 2 × 2 m, with a spacing of 1 m between the plots. Thereafter, planting holes were made at a spacing of 40 × 15 cm in each plot.

Experimental design

Field experiments were conducted in a randomized complete block design (RCBD) across five smallholder farms in each of the three counties (Embu, Tharaka Nithi and Kitui). Three cowpea varieties (K80, M66, KVU) were tested with four treatments: indigenous rhizobia, commercial rhizobia, a consortium of native and commercial rhizobia and an uninoculated control. Each treatment was replicated three times. The study was a continuation of previous study; hence, the indigenous isolates used were previously isolated by Muindi *et al*. (2017). Cowpea varieties (K80, M66, KVU), recommended for Kenyan arid and semi-arid regions, were sourced from the Kenya Agricultural and Livestock Research Organization. Commercial rhizobia (USDA 3456, known as Biofix) were obtained from MEA Limited Kenya.

Inoculum application, planting and management

The rhizobia Inoculum was coated with cowpea seeds asceptially and in separate basins under a shade. Native rhizobia in nutrient broth were applied with sugar as a sticker as described by Nyaga and Njeru (2020). Additionally, the commercial rhizobia (Biofix) were applied to the cowpea seeds as described by the manufacturer (100 g of inoculum per 15 kg of seeds). Two seeds were planted per hole, with control plots planted first to minimize cross-contamination. Weeds were controlled occasionally and gapping was performed after germination. Pest control involved spraying Aceprid 20 WSP for thrips during flowering and Evisect for whiteflies. Plants were rain-fed, with no additional irrigation.

Field sampling

At the flowering stage (six weeks after germination), three plants per plot were randomly uprooted. The root noudles were detached, counted, wrapped in absorbent paper and placed in storage bags. Plant height was measured and leaves were counted. Samples were transported to Kenyatta University for air-drying, followed by determination of nodule dry weight and shoot dry weight.

Harvesting

At physiological maturity (90 days after planting), three random plants per plot were sampled for stover dry weight, number of pods, 100-seed weight and total seed dry weight. Pods and seeds per pod were counted and stored separately. Similarly the stovers were separated for air-drying in the laboratory. The laboratory and greenhouse experiments were conducted at Kenyatta University (geographical position: 1.18°S 36.93°E).

Table 1: Description of study sites in Eastern Kenya.

Parameter	Embu county	Tharaka Nithi county	Kitui county
Latitude/longitude	0.27″S/37.65″E	0°17′60″N/38°00′13″E	1.19″S/37.86″E
Elevation (m above sea level)	>1174	600-1500	400-1800
Rainfall (mm/year)	1485	700	881
Soil texture	Sandy loam	Sandy loam	Sandy loam/sandy clay loam
% Clay	12-18	12-18	24
% Sand	44-76	44-76	64
% Silt	8-12	8-12	14
% Total organic carbon	1-2.07	1-2.07	0.63
% Total nitrogen	0.1-1.8	0.1-1.8	0.07
Phosphorus (ppm)	35	28	20
Potassium (ppm)	1.02	0.24	1.72
Soil pH	5.7-7.1	5.7-7.1	6.27

Isolation of rhizobia from the cowpea nodules

For rhizobia isolation, nodules from three random plants per plot were collected at flowering. After washing roots to remove soil, nodules were detached, stored in sterile vials with cotton wool and silica gel and transported to the lab. In the lab, nodules were washed, soaked in sterile distilled water (1-2 hours) for softening and surface-sterilized with 95% ethanol (to remove air and reduce surface tension) followed by 3% hypochlorite (2-4 minutes) and five sterile distilled water rinses. Using sterilized forceps and petri dishes, nodules were crushed in 1 mL sterile distilled water. A loopful of the suspension was streaked in triplicate on Congo red YEMA plates, sealed with Parafilm and incubated at 30°C for 48 hours. Pure colonies were obtained by repetitive subculturing and stored as stock cultures on YEMA slants in McCartney bottles at 4°C.

Morphological and biochemical characterization

Phenotypically pure isolates were identified based on culture, microscopic and biochemical characteristics on YEMA medium. Colony characteristics (size, color, shape, elevation, transparency) were observed after incubation. Gram staining was performed on typical rhizobia colonies, as described by Panicker *et al*. (2023). For biochemical characterization, isolates were re-streaked on YEMA containing bromothymol blue (BTB) to assess acid/alkaline production and on Congo red YEMA medium to observe dye absorption after 48-72 hours at 28°C.

DNA extraction and PCR amplification

Pure rhizobia colonies from YEMA agar plates were used for DNA extraction via the CTAB followed by phenol-chloroform method as described by (Gautam, 2022). The 16S rRNA gene was amplified in a 30 µL PCR reaction using 27F and 1492r primers, containing PCR water, 10X buffer, dNTPs, Tween 20, Taq DNA polymerase and 1.5 µL DNA template. Amplification involved 30 cycles of denaturation (95°C), annealing (55°C) and extension (72°C), with initial (5 min at 95°C) and final (5 min at 72°C) extension (Pichler *et al*., 2018).

Gel electrophoresis

The polymerase chain reaction products were analyzed by gel electrophoresis using 2% agarose gel and amplified DNA Electrophoresis was performed using 1× TBE buffer and SYBR-green staining dye. The gel was visualized using a trans-illuminator and an image captured using a digital camera. The electrophoresis was performed at 120 volts for 50 minutes.

Statistical analysis

All data on root dry weight (RDW), shoot dry weight (SDW), total seed weight and 100-seed weight were subjected to analysis of variance (ANOVA) using the General Linear Models Procedure of SAS software version 9.1. and means tested for significance using Least Significance Differences (LSD) at p<0.05.

RESULTS AND DISCUSSION

Effect of rhizobia on cowpea growth and yield

Inoculated cowpea plants consistently outperformed uninoculated controls across growth parameters, demonstrating the efficacy of rhizobia inoculation in enhancing plant performance. For instance, shoot dry weight varied significantly (p=0.0014), with indigenous rhizobia (VD) producing higher weights (16.53 g/plant) compared to commercial rhizobia (VM), consortium (VDM) and uninoculated control (VC). Similarly, cowpea height showed notable increase with indigenous inoculation recording the highest plant height (55.62 cm) compared to VM (50.77 cm), VDM (44.23 cm) and VC (32.39 cm) (Table 2). These results confirm that treatment, cowpea variety and regional differences interact strongly showing that genotype and environment influences cowpea growth.

Plant height, leaf number and shoot dry weight were markedly higher in K80 compared to KVU and M66 varieties, showing that K80 variety had superior compatibility with indigenous rhizobia (Table 2). Furthermore, regional variations in nodule formation and plant parameters were attributed to differences in critical soil nutrients like phosphorus, which is vital for nodule development and leghemoglobin content (Wang *et al*., 2020; Mathenge *et al*., 2019). Regional disparities were evident, with Embu outperforming Tharaka Nithi and Kitui (p = 0.0001), consistent with earlier studies showing that soil nutrient profiles affect nodulation (Mathenge *et al*., 2019).

Yield parameters

Indigenous rhizobia (VD) and consortium treatments (VDM) significantly increased cowpea yields (2933 kg ha^{-1} and 2807 kg ha^{-1}, respectively) compared to Commercial (VM) and control (VC) (2604 kg ha^{-1} and 2547 kg ha^{-1}, respectively) (Table 2). This indicates superior adaptation of native strains to local soil and climate, enhancing competitiveness and symbiotic efficiency (Nyaga and Njeru, 2020). The reintroduction of these adapted indigenous strains appears to bolster their population in the soil, fostering more efficient symbiosis, improved nutrient availability and consequently, greater vegetative growth and seed yield (Kyei-Boahen *et al*., 2017; Wolde-meskel *et al*., 2018; Sapna and Sharma, 2021).

Morphological and biochemical characteristics of rhizobia

The isolated rhizobia exhibited diverse morphological traits, such as mucoid colony texture and Gram-negative reactions, consistent with previous reports (Ondieki *et al*., 2017). Variations in colony color, elevation and biochemical reactions on Congo Red and bromothymol blue media revealed the heterogeneity among isolates, underscoring the genetic diversity of rhizobia from different agroecological zones.

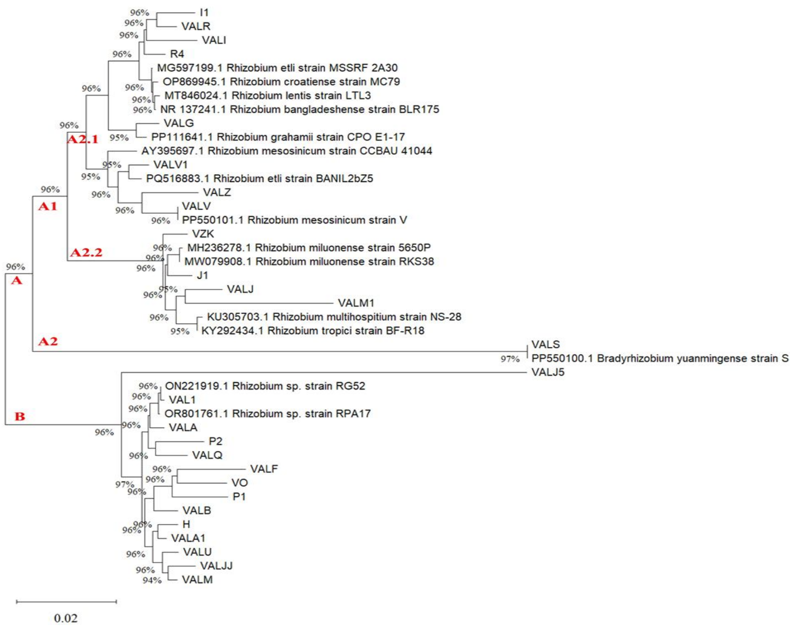
Genetic diversity of native cowpea-nodulating rhizobia

Phylogenetic analyses clustered the isolates into two main clades, representing species such as *Rhizobium etli, R. lentis*

Table 2: Effect of rhizobia on cowpea yield.

Treatments	Height (cm)	Leaf number	Nodule number	Nodule weight (g)	Shoot dry weight (g)	Yield (kg-ha)	100 seeds dry weight (g)
Regions							
Embu	52.07±0.92^{a}	43.13±0.97^{a}	10.97±0.44^{a}	0.06±0.00^{a}	21.86±0.60^{a}	4237.52±154.85^{a}	12.56±0.19^{a}
Tharaka	48.46±0.79^{b}	26.52±0.93^{b}	6.32±0.30^{b}	0.04±0.00^{b}	12.25±0.58^{b}	3338.20±203.98^{b}	12.71±0.18^{a}
Kitui	36.73±0.60^{c}	11.64±0.41^{c}	5.11±0.26^{c}	0.03±0.00^{b}	10.16±0.24^{c}	582.18±52.11^{c}	10.15±0.17^{b}
Varieties							
K80	49.10±0.96^{a}	30.32±1.68^{a}	7.20±0.34^{a}	0.04±0.00^{a}	15.78±0.69^{a}	3058.28±210.69^{a}	11.03±0.17^{b}
M66	44.89±0.92^{b}	20.85±1.05^{b}	7.56±0.43^{a}	0.05±0.00^{a}	14.08±0.66^{b}	2496.95±179.44^{b}	11.90±0.20^{b}
KVU	43.26±0.83^{c}	20.18±0.95^{b}	7.64±0.40^{a}	0.04±0.00^{a}	14.41±0.50^{ab}	2614.58±177.93^{b}	12.50±0.21^{a}
Treatments							
VD	55.62±0.98^{a}	29.93±1.86^{a}	7.96±0.48^{a}	0.05±0.00^{a}	16.53±0.85^{a}	2807.40±230.04^{b}	11.75±0.24^{a}
VM	50.77±0.72^{b}	23.55±1.47^{b}	7.06±0.41^{a}	0.04±0.00^{a}	14.47±0.63^{b}	2547.58±206.96^{c}	11.81±0.23^{a}
VDM	44.23±0.64^{c}	22.83±1.43^{b}	7.62±0.52^{a}	0.04±0.00^{a}	13.96 ±0.79^{b}	2933.21±236.74^{a}	11.74±0.24^{a}
VC	32.39±0.62^{d}	18.86±1.06^{c}	7.22±0.39^{a}	0.04±0.00^{a}	14.06 ±0.58^{b}	2604.90±205.47^{c}	11.92±0.21^{a}
P value of the main effect and their interactions							
Region	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Treatment	<.0001	<.0001	0.3782	0.7949	0.0014	0.3081	0.9127
Varieties	<.0001	<.0001	0.6325	0.7536	0.0201	0.0106	<.0001
Treatment* Region	<.0001	<.0001	0.8828	0.9523	<.0001	0.0035	0.4169
Varieties* Region	<.0001	<.0001	0.6737	0.8574	<.0001	<.0001	0.0057
Treatment* Varieties	<.0035	<.0001	0.0776	0.2032	0.0134	0.0040	0.8552
Treatment*Varieties *Region	<.0261	<.0001	0.2787	0.2807	0.0006	<.0001	0.4405

VD- Indigenous; VM- Commercial; VDM- Consortium; VC- Control; Means with the same superscript letters within the same column are not statistically significant.



Evolutionary distances were computed by the p-distance method and are in the units of the number of base differences per site. Cluster levels are indicated by labels in red at the branches.

Fig 1: Phylogenetic tree showing the genetic relationship between isolates based on the neighbor-joining method.

Table 3: Effect of cowpea nodulating rhizobia cross-inoculation on common beans.

	SDW	RDW	NN	NDW
Varieties				
GLP2	1.46±0.11[a]	0.787±0.066[a]	8.615±2.407[a]	0.021±0.006[a]
GLP92	1.098±0.075[b]	0.569±0.041[b]	7.205±1.263[a]	0.026±0.005[a]
Treatments				
A1	1.407±0.158[abc]	0.852±0.121[ab]	10.000±2.017[bc]	0.044±0.016[ab]
G	0.780±0.059[c]	0.598±0.071[ab]	4.667±0.954[c]	0.007±0.003[de]
I	0.898±0.131[bc]	0.575±0.077[ab]	0	0
I1	1.517±0.403[ab]	0.792±0.111[ab]	17.667±7.749[ab]	0.044±0.024[ab]
J	1.977±0.296[a]	0.800±0.603[ab]	19.167±8.976[ab]	0.038±0.016[bc]
M1	1.112±0.225[bc]	0.810±0.159[ab]	10.667±4.964[bc]	0.046±0.019[ab]
R	1.102±0.113[bc]	0.445±0.054[ab]	4.000±2.066[c]	0.027±0.012[bcd]
S	1.208±0.201[bc]	0.650±0.114[ab]	5.000±2.352[c]	0.013±0.003[cde]
V	0.967±0.189[bc]	0.673±0.137[ab]	0.167±0.167[c]	0
ZK	1.262±0.067[abc]	0.648±0.129[ab]	6.000±2.733[c]	0.006±0.002[de]
Negative cntrl	0.945±0.095[bc]	0.665±0.119[ab]	0	0
Positive cntrl	1.410±0.226[abc]	0.323±0.031[ab]	0	0
P-values				
Treatment	<.0001	0.0756	<.0001	<0.0001
Variety	<.0001	0.0042	0.2584	0.0716
Treatment*Variety	<.0001	0.3602	<.0001	<.0001

A1- *Rhizobium sp*, G- *R. grahamii*, I- *R ,lentis*, I- *R. etli*, J- *R. multihospitium*, M1- *R. tropici*, R- *R. bangladenshense*, S- *Bradyrhizobium yuanmingense*, V- *R. mesosinicum*, ZK- *R. miluonense* SDW- Shoot dry weight; RDW- Root dry weight; NN- Nodule number; NDW- Nodule dry weight; Means with the same superscript letters within the same column are not statistically significant.

and *R. tropici* (Fig 1). Genetic diversity was significantly high in Kitui (nucleotide diversity Pi=0.07048), reflecting its broader environmental variability compared to Embu and Tharaka Nithi. Overall, the observed diversity suggests strong local adaptation of rhizobia populations to distinct agroecological zones.

Cross-inoculation effects on common bean

Cross-inoculation of common beans with cowpea native rhizobia led to significant increase in common bean root dry weight, shoot dry weight, nodule number and nodule dry weight (Table 3). Although rhizobia are typically host-specific, certain cowpea isolates (*e.g.*, J and I1) showed strong cross-compatibility with common bean, revealing useful symbiotic flexibility (Gunnabo *et al*., 2019). This challenges the conventional understanding of *Phaseolus vulgaris* nodulation and suggests potential for developing broad-spectrum inoculants. The genetic diversity observed among the isolated native rhizobia, including species like *Rhizobium etli* and *R. tropici* (Odori *et al*., 2020), further supports cowpea's promiscuous nature and highlights the potential for these locally adapted strains to benefit multiple legume crops, improving N fixation and overall agricultural productivity in the region.

CONCLUSION

Rhizobia inoculations significantly improved cowpea height, nodule number, nodule weight, shoot dry weight, total seed weight and 100-seed weight. However, these parameters differed between the treatments, cowpea varieties and the regions. The isolated rhizobia differed genetically between the regions and included *Rhizobium etli, Rhizobium lentis, Rhizobium bangladeshense, Rhizobium multihopitium, Rhizobium tropici, Rhizobium graham, Rhizobium mesonicum, Rhizobium* sp. and *Rhizobium yuanmingense*, highlighting their adaptation to local soil and climatic conditions. Under greenhouse conditions, crossinoculation of rhizobia isolated from cowpea on common beans resulted in a considerable increase on growth and yield parameters. This finding challenges the traditionally narrow host range of rhizobia associated with common bean and suggests the need for broader application of cowpea-derived rhizobia as biofertilizers in smallholder mixed-legume systems. Future research should investigate the long-term effectiveness of these native rhizobia inoculants, optimize their application methods and explore the specific genetic and molecular mechanisms underlying their observed symbiotic promiscuity with common bean varieties.

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Author's contribution

Valentine Mburu, Ezekiel Mugendi Njeru and John Maingi designed the project. Valentine Mburu and Mourine Mutai conducted the experiments, analyzed the data and prepared the manuscript. All authors reviewed the manuscript and approved it for publication.

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Conflict of interest

The authors declare no conflict of interest.

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