# Morphological assessment and effectiveness of indigenous rhizobia isolates that nodulate *P. vulgaris* in water hyacinth compost testing field in Lake Victoria basin

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## ABSTRACT

**Aims:** The study was aimed at isolating, identifying and assessing the effectiveness of indigenous rhizobia nodulating *P. vulgaris* in Lake Victoria Basin (LVB).
**Study design:** Randomized complete block design.
**Place and Duration of Study:** Soil and nodule samples were collected from Kisumu (Kenya); Kabanyolo (Uganda) and Nyabarongo (Rwanda). Field experiments: Kisumu (Kenya). Lab and greenhouse experiments: Department of Plant and Microbial Sciences Kenyatta University (Kenya) and Makerere University (Uganda). Research was carried out between January 2012 and April 2013.
**Methodology:** Rhizobia were isolated from nodules obtained from *P. vulgaris* (rose coco variety) plants planted in the LVB water hyacinth compost trial fields and whole soil trapping experiments in the greenhouse using soil obtained from the LVB. The isolates were identified using morphological characteristics. Isolates from each group were used in authentication using the infection technique.
**Results:** One hundred and twenty eight isolates were obtained from the trapping experiments and placed into nine groups based on their morphological characteristics. Four hundred and seventy two isolates were obtained from the nodules of the *P. vulgaris* grown in soils amended with water hyacinth compost and were placed into sixteen groups. The isolates varied in their morphological characteristics. There was a significant difference in the infectiveness and effectiveness of the representative rhizobia isolates.

**Conclusion:** The studies revealed that rhizobia isolates from Lake Victoria are different morphologically. Authentication experiments, confirmed that the majority of the isolates were rhizobia due to their ability to infect the host plant *P. vulgaris*. All representative isolates varied in their ability to infect and fix nitrogen. Isolates that are more effective compared to the commercial *Rhizobium leguminosarum biovar phaseoli* strain 446 were identified in this study. The effective indigenous rhizobia have therefore the potential of being sources of inocula for *P. vulgaris*.

*Keywords: Rhizobia; Morphological characteristics; Authentication; Phaseolus vulgaris.*

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## 1. INTRODUCTION

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Lake Victoria is the second largest fresh water lake in the world and occupies about 69000 $km^2$. The Lake Victoria Basin (LVB) has an area of approximately 251,000 $km^2$ [1]. Twenty two percent of the catchment area is in Kenya, 11 % in Rwanda, 16 % in Uganda, 7 % in Burundi and 44 % in Tanzania [2]. According to Albinus et al. [3] LVB is characterized by high human population growth and currently the population is more than 40 million, with estimated 30 % of the total population living in the three riparian countries; Kenya, Tanzania and Uganda. Most of the people in this region are subsistence farmers who rely on natural rainfall for crop production and they mainly cultivate maize (*Zea mays*) and common beans [4].

Continued increase in population, poor agricultural and livestock production methods, and deforestation are major causes of land degradation and reducing productivity in the LVB [5,6] [5, 6]. To boost food production from the dilapidated farms, farmers are encouraged to use manure or inorganic nitrogen fertilizers. Nitrogen requirements in the soil are usually higher as compared to other major soil nutrients for sustainable food production [7]. Studies have shown that despite availability of other nutrient sources to enhance nitrogen in soil for improved crop yield, chemical fertilizers have been prioritized as a solution to nutrient deficiencies in the soil [7,8]. Too much use of nitrogen fertilizer for agricultural production has been reported to contribute to greenhouse gas emissions, reduce water quality, reduce biodiversity and is potential health hazard [9]. Agricultural runoff is a major source of high nitrogen loads in Lake Victoria and it accounts for 75 percent of the total nitrogen flow into the lake from the lakes catchments, with most of the nutrients being deposited into the lake during the wet season of the year [10,11,12]. Increased inflow of agricultural runoff into Lake Victoria has resulted into increase in nutrient concentrations and turbidity and reduction of dissolved oxygen [13]. This in turn has led to algae blooms, infestation of waterweeds especially the water hyacinth, fish kills and water- borne diseases [13]. The cost of inorganic fertilizers has also been in upward trend making it unaffordable by many small scale farmers [14]. To enhance food crop production, there is need to adopt cheaper and environmentally friendly means of improving soil fertility [7,14].

Due to the dangers encountered as a result of inorganic fertilizers production and use, this calls for urgent measures for alternative plant nutrient sources that are environmentally

friendly [15]. Other than the use of inorganic fertilizers in crop production biological di-nitrogen fixation using rhizobia has been beneficial [16]. Rhizobia have the ability to fix $N_2$ through their symbiotic relationship with leguminous plants [17]. Biological nitrogen fixation (BNF) is a climate change resilient farming system and boosts adequate management of soil, water and biodiversity and is also cost effective [18,19]. Leguminous plants also have the ability to contribute to increased soil nitrogen and potentially lead to increase in yields of succeeding and associated non-nodulating plants via symbiotic nitrogen fixation [20]. BNF is also important especially in regions with great farmland pressure and where fallow system is not possible [19, 21].

*Phaseolus vulgaris* L. (common bean) is an important legume for human nutrition and a major source of protein, complex carbohydrates, folic acid and dietary fiber [22,23]. According to FAO *Phaseolus vulgaris* is also a source of steady income for scores of rural households [24]. *Phaseolus vulgaris* yield is low in East Africa, mainly due low soil fertility with most of the soils having low available nitrogen and phosphorus [25]. Like other leguminous plants this plant is able to establish nitrogen fixing symbiotic relationship with rhizobia, which can improve the crop yield [22,26]. *P. vulgaris* is described as promiscuous in its symbiotic interactions, because it has the ability of nodulating to nodulate with a diversity of rhizobial species [27, 28]. Rhizobial species nodulating *P. vulgaris* include *Rhizobium leguminosarum* bv. *phaseoli*, *R. gallicum (*bv. *phaseoli and* bv. *gallicum)*, *R. tropici*, *R. giardinii (*bv. *phaseoli* and *bv. giardinii)* and *R. etli* bv. *Phaseoli* [27].

It has also been reported that rhizobia, which are indigenous to African soils, nodulate and fix nitrogen in common bean and this process is important for soil improvement and improved crop yield [29]. Despite the common bean being able to have symbiotic relationship with rhizobia, the interactions are not always effective; however strains that are adapted to the local environment have been shown to be more effective in nitrogen fixation [30, 31].

Knowledge on indigenous rhizobia nodulating *P. vulgaris* in the LVB in the soil amended with water hyacinth compost is limited. The objectives of this study were to isolate, identify and group the isolates based on their cultural characteristics, and to carry out authentication of the isolates.

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## 2. MATERIALS AND METHODS

### 2.1 Study area

Field experiments were carried out at Korando B sub-location in Kisumu (Kenya), Kabanyolo (Uganda) and Nyabarongo (Rwanda), laboratory experiments at Kenyatta and Makerere Universities and greenhouse experiments at Kenyatta University.

### 2.2 Soil sampling

In Kenya, farm A (S 00° 05.404'; E 034° 41.862'), Farm B (S 00° 05.120'; E 034° 41.613'), farm C (S 00° 05.325'; 034° 41.796') and farm D (S 00° 05.167; E 034° 42.084'), all in Korando B sub-location in Kisumu County, Kenya were used. Sampling was carried out diagonally and across the farm at 20 points in each plot. Before collecting the soil, organic matter on the soil surface was cleared. Soil was dug to a depth of 20 - 30 cm from the soil surface. A kilogram of homogenous soil sample from each cross section was packed independently. The soils were collected aseptically to avoid cross contamination. The soil was then transported to Kenyatta University lab for storage at a temperature of 4 ºC.

### 2.3 Soil analyses

Soil samples from four farms were analyzed in Makerere University lab Uganda according to the procedure described by Okalebo et al. [32].

### 2.4 Greenhouse rhizobia trapping experiments

In the greenhouse soil collected from the different points in each locality in Kenya, Uganda and Rwanda was mixed to form a homogenous composite sample for each plot. The soil samples were then potted in six different sterilized pots accommodating approximately one kilogram of soil. Rose coco bean that is mainly planted in the study locality was used as the trapping host. The bean seeds were surface sterilized using 3 % sodium hypochlorite and pre-germinated on a nutrient free agar media before planting. Two seedlings were planted per pot after three days pre-germination of the seeds. The pots were arranged in a randomized complete block design. Watering was carried out at one day interval because of the high water holding capacity of the soils. Nodulation assessment on the plants was carried out 35 days after planting. The roots were carefully washed and the nodules were detached and wrapped with absorbent tissue paper to dry at room temperature. Trapping experiments were also carried out in Uganda and Rwanda.

## 2.5 Rhizobia trapping in the field

Nodules were obtained from rose coco variety of *P. vulgaris* plants from the four farms (Farm A, B, C and D) in Korando B Sub-location in Kisumu (Kenya) during the long rains season starting from March 2012. Three bean plants were sampled for nodule analysis from plots which had been treated with the following: water hyacinth compost made using cattle manure and Effective Microorganisms (EM), negative control in which the water hyacinth was treated with water, DAP (Commercial fertilizer) and soil with no amendment (control). The harvesting of the plants was carried out at the onset of flowering. The roots were carefully washed, nodules detached and wrapped with absorbent tissue paper to dry.

## 2.6 Isolation of rhizobia

Nodules representing each host plant from all the soil treatments were selected from the preserved nodules and those from trap experiment in the greenhouse. They were put in sterilized distilled water and let to imbibe water for one hour. They were then rinsed with distilled water and dipped for 5 seconds in 95 % ethanol to reduce the surface tension and remove air bubbles from the tissues. The nodules were then sterilized by dipping them in 3 % (v/v) sodium hypochlorite solution for 4 minutes. They were then rinsed in five changes of sterile distilled water and crushed with a sterile glass rod in a drop of sterile distilled water. A loop full of the nodule suspension was streaked onto Yeast-Mannitol agar (YEMA) plates containing Congo Red and incubated at room temperature in the dark and observations made after three days. After five days of incubation well isolated colonies were streaked on YEMA plates containing Congo Red. The isolates were grouped using procedure described by Odee et al. [33]. The morphology of the different colonies was recorded. The morphological characteristics used were; colony elevation, colony consistency, colour, texture, size of the first independent colonies and shape of the margins. The isolates were also evaluated for their ability to change the pH of the media by growing them on YEMA media substituted with Bromothymol blue (BTB).

## 2.7 Rhizobia authentication through re-inoculation of *P. vulgaris*

Representative isolates for both whole soil trapping experiment and on farm trapping were tested to confirm their nodule forming ability on the host legume under bacteriologically controlled conditions. *P. vulgaris* seeds were selected for uniformity in size, shape and colour and then surface-sterilized with a 3.0 % (v/v) sodium hypochlorite for 6 min, followed by rinsing in five changes of sterile distilled water. The sterilized seeds were pre-germinated in kilner jars containing damp sterile vermiculite at a temperature of 28 °C. The seedlings

were then transplanted aseptically into Leonard jar assemblies [34,35]. Three seedlings were planted into each Leonard jar and then later thinned to two. Four replicates were used for each treatment. The rooting medium comprised of washed nutrient free vermiculite with a pH of 6.8 [36]. The seedlings were maintained for eight days in Leonard jar assemblies before they were inoculated with 1 ml of the representative rhizobia isolates cultured in YEMA broth for three days. The jars were arranged in a randomized complete block design in a greenhouse under non-sterile conditions. The seedlings were irrigated with sterile nitrogen free nutrient solution containing in g/L: $CaCl_2$ 0.1, $MgSO_4.7H_2O$ 0.12, $KH_2PO_4$ 0.1, $Na_2HPO_4.2H_2O$ 0.15, Ferric citrate 0.005, and 1.0 ml of trace elements stock solution [34]. The trace elements stock solution contained: $H_3BO_3$ 2.86, $MnSO_4.7H_2$ O 2.03, $ZnSO_4.7H_2O$ 0.22, $CuSO_4.5H_2O$ 0.08, and $NaMoO_2.2H_2$ O 0.14 in g/L. The pH of the nutrient solution was adjusted to 6.8 with NaOH. Jars of uninoculated seedlings were used as negative control and inoculated jars with a commercial rhizobia strain 446 were used as positive control. Nodule formation was recorded after 45 days.

The plant roots were carefully washed with tap water to remove vermiculite and then the attached wick carefully removed taking care not to destroy the roots and nodules. The plants were scored for the presence or absence of nodules and the number of nodules per plant. Presence of a single nodule in a Leonard jar for any plant was considered as a confirmation that the isolate is rhizobia [37]. The nodules were then wrapped in tissue paper and stored at room temperature. Shoots were separated from roots and separately oven-dried at a temperature of 60 °C until they achieved constant dry weight and their respective biomass was determined according to the procedure by Bala et al. [38].

### 2.8 Data analysis

The data on the root, nodule and shoot dry matter and number of nodules were analysed using Analysis of Variance (ANOVA) with SPSS computer software version 11.5. Tukey's test at 5 % probability level was used to separate means. Morphological data of the isolates was coded into numerical values and used for cluster analysis. The phenogram was based on a hierarchical cluster analysis using the squared Euclidean distance similarity and single linkage (nearest –neighbor) procedures using SPSS software version 16.

## 3. RESULTS AND DISCUSSION

### 3.1 Soil analysis (Please avoid to have title in the end of your page)

The soil pH was slightly below the critical level in farm C and farm B in Kenya, all farms in Rwanda, MUARIK, farm 1 and farm 2 in Uganda, within the critical level in farm 3 in Rwanda and above the critical level in farm A (Table 1). SOM ( soil organic matter) and N was below the critical level in all the soils except the one from farm 3 in Uganda. Phosphorus content was below the critical level (15 mg $kg^{-1}$) in all the sites but high in farm A (23.7 mg $kg^{-1}$). Calcium (Ca) was higher compared to the critical value in the soil samples from all the farms except the soils from farm 1 and 2 (Rwanda) and MUARIK farm (Uganda).

**Table 1. Soil characteristics of experimental sites compared with critical values for East African soils**

| SITE | pH | SOM | N | Av. P | K | Ca | Na | Textural class |
|---|---|---|---|---|---|---|---|---|
| | | % | | mg $kg^{-1}$ | Exchangeable (cmol. $kg^{-1}$) | | | |
| **KENYA** | | | | | | | | |
| Farm A | 6.4 | 1.89 | 0.11 | 23.7 | 2.82 | 14.0 | 0.09 | SL |
| Farm B | 5.1 | 1.21 | 0.07 | 7.8 | 0.37 | 5.0 | 0.03 | SL |
| Farm C | 5.4 | 1.21 | 0.08 | 11.0 | 0.49 | 7.6 | 0.03 | SL |
| **RWANDA** | | | | | | | | |
| Farm 1 | 4.9 | 2.09 | 0.09 | 9.2 | 0.20 | 3.2 | 0.03 | SL |
| Farm 2 | 5.2 | 1.99 | 0.09 | 8.8 | 0.24 | 3.4 | 0.03 | SL |
| **UGANDA** | | | | | | | | |
| Uganda (MUARIK) | 5.0 | 3.2 | 0.08 | 4.75 | 0.50 | 3.3 | 0.3 | SC |
| Uganda (Farm 1) | 5.3 | 3.12 | 0.13 | 10.95 | 0.16 | 7.35 | 0.16 | SCL |
| Uganda (Farm 2) | 5.4 | 2.4 | 0.13 | 10.84 | 0.27 | 6.09 | 0.16 | SCL |
| Uganda (Farm 3) | 5.5 | 2.96 | 0.13 | 10.5 | 0.16 | 7.3 | 0.08 | SCL |
| †Critical value | 5.5 | 3 | 0.25 | 15 | 0.22 | 4.0 | <1 | |

† Okalebo *et al*. [32] ; SOM, Soil organic matter; SL, Sandy loam; SCL, Sandy clay loam; N, Nitrogen, K, Potassium, Ca, Calcium, Na, Sodium, Av. P, average phosphorus.

### 3.2 Whole soil indigenous rhizobia trapping

There was a significant difference in the nodulation of *P. vulgaris* between soil obtained from Farm B and farm C compared to farm A (Table 2). Farm A had the highest mean nodulation

of 73.58 nodules per plant (Table 2). There was no significant difference on mean nodulation between farm B and farm C. Beans grown on soils from farm B had the lowest mean nodulation; however beans grown on soil from farm B appeared greener as compared to those in soils from farm C which had highest mean nodulation compared to the farm B (Fig. 1). It was also observed that there was no significant difference on root dry weight of the beans grown on soils from all the farms. There was no significant difference on shoot dry weight between bean plants grown in soils from farm C and farm A and also Farm C and farm B.

**Table 2. Nodulation and bean plant biomass in the greenhouse**

| Soil | Elevation (Masl) | Mean nodule number | Root dry weight (RDW) (g) | Shoot dry weight (SDW) (g) |
|---|---|---|---|---|
| **Farm A** | 3762 | 73.58b[+] | 0.16a[+] | 1.42b[+] |
| **Farm B** | 3773 | 14.70a | 0.63a | 0.74a |
| **Farm C** | 3777 | 23.82a | 0.21a | 0.82ab |
| **P. value** | | 0.000 | 0.301 | 0.040 |

[+]Values followed by the same letters within the columns are not significantly different from each other according to Tukey`s Honest Significant Difference (HSD) at 5 % level.

The observed results can be attributed to the soil characteristics from each site (Table 1).

There was no recent history of bean crop cultivation in farm B. Long duration without common bean cultivation in the farm B could also have contributed to the low nodulation. Studies have demonstrated that continuous cultivation improves build-up of rhizobia in soil and increases nodulation [39]; however other factors like soil pH and mineral composition of the soil could also have contributed to the low nodulation in this soil.

Soil characteristics varied from farm to farm (Table 1) and this could help in interpretation of the observed differences in mean bean nodulation and total biomass (Table 2; Fig 1). Levels of soil pH, temperature, osmotic stress and nutrient availabilty influence rhizobia ability to get into symbiotic interaction with legumes [40]. Nodulation is also affected by absence of indigenous related legumes, soil texture and heavy metals [41,42].

Lower Ca, lower P and the acidic pH in the soil samples from farm C and farm B could have contributed to lower nodulation ( Table 1). Higher Ca and near basic pH in in the soil from

Farm A could have contributed to the higher nodulation compared to farm B with the lowest Ca levels and acidic pH. According to Mohammadi et al. [43] sufficient calcium levels and suitable soil pH are required for good nodulation in legumes, because acidic conditions and low calcium levels inhibit formation of nodules.

High available P(phosphorus) in the soil is reported to stimulate nodulation in legumes overcoming the inhibitory effects of high N on nodulation [44]. Phosphorus is also important in the nutrition of legume crops and improves the root and shoots growth, and this influences *Rhizobium* efficiency [45]. Higher P concentrations could have contributed to the high mean nodulation observed in soils obtained from farm A as compared to to the farm B and farm C.

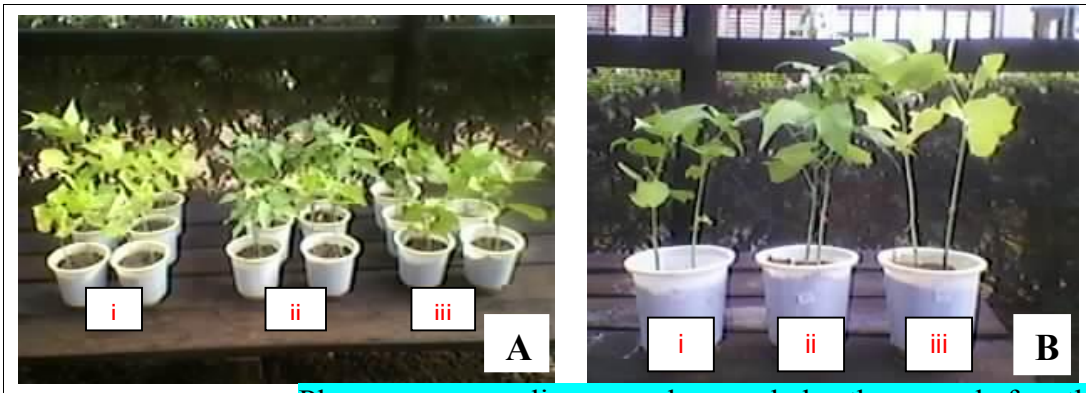


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**Fig 1. Effect of soils from farms in Korando B sub-location Kisumu, Kenya on growth and nodulation of *P. vulgaris.* A, (i, Farm *C*. ii; Farm A; iii, Farm B) *P. vulgaris* plants after 30 days of growth in the greenhouse. B, (i, Farm B pot 4. ii; Farm A; pot 4) iii, Farm C pot 3) individual pots showing effect of different soils on growth and nodulation of *P. vulgaris*. Differences were noted in color and height of the plants as per the treatment.**

### 3.3 Morphological characterization of rhizobia isolates

A total of 128 pure rhizobia isolates were obtained from the whole soil trapping nodules out of these 118 of the isolates were from Kisumu (Kenya), five isolates from Uganda and five isolates from Rwanda site. The 128 pure nodule isolates obtained fall into nine groups (Table 3; Fig. 2). A total of 472 isolates were obtained from the on farm trapping experiment grouped into 16 groups based on morphological characteristics (Tables 4 and 5, Fig. 3). According to Loureiro et al. [46], it is better to study rhizobia diversity by isolating rhizobia from root nodules collected from field trapping experiment as opposed to greenhouse trapping experiments, which possibly explains why more rhizobia isolate groups where recovered from the on-farm trapping experiments. The colony elevation was convex, domed, or raised (Tables 3 and 4).

The colony consistency was either gummy, firm gummy and soft gummy for colonies with excessive extracellular polysaccharide (EPS) production. Colony appearance was either

316 opaque or translucent and the color was white, creamy, milky, watery, or curdled milky
317 (Table 3, Fig 2 and 3). Most of the isolates from whole soil trapping experiments were
318 categorized in group VI and VII with 31.36 % and 42.37 % of all the isolates respectively
319 (Table 3). The bulk of the isolates obtained from the nodules in farm trapping after compost
320 treatment were in two main groups Group IV and VII that had the highest percentage of
321 isolates from all the farms (Table 5). All the isolates acidified the media substituted with BTB
322 turning it to yellow in color; a characteristic of fast growing rhizobia [47]. Prior studies have
323 shown that common beans nodulates mainly with fast growing rhizobia grouped under *R.*
324 *gallicum* and *R. giardini* [48], *R. leguminosarum* bv. *Phaseoli,* [49], *R. tropici* [50] and *R. etli*
325 [51]. The recorded morphological traits of most of the pure isolates are typical of rhizobia
326 [52]. Rhizobia isolates in group iii and XV, obtained from on farm trapping, however were
327 yellow in color; a characteristic that has been reported for fast growing rhizobia [53].
328 Moreover, rhizobia are known to produce surface polysaccharides including
329 exopolysaccharides (EPSs) and lipopolysaccharides (LPSs) that are believed to help
330 restrain host defense reactions [54].
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**Table 3. Whole soil trapping rhizobia isolates from nodules of *P. vulgaris* plants before the application of water hyacinth compost**

| Isolate characteristics | Isolate groups | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | i | ii | iii | iv | v | vi | vii | viii | ix |
| Margin | e | e | e | e | e | e | e | e | e |
| Gram reaction | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve |
| BTB reaction | y | y | y | y | y | y | y | y | y |
| Congo red absorption | crna | crna | crna | crna | crna | crna | crna | crna | crna |
| Colour | mw | cy | mw+a | cm +b | ws | w | mw+o | cy | p |
| Transparency | o | o | o | t | o | t | t | o | o |
| Cell shape | rod | rod | rod | rod | rod | rod | rod | rod | rod |
| Nature of the colony | shiny | shiny | shiny | shiny | shiny | shiny | shiny | shiny | shiny |
| Colony ɵ (mm) | 4 | 3.5 | 4 | 2 | 5 | 2 | 3.5 | 0.5 | 0.5 |
| Elevation | cvx | cvx | cvx | cvx | cvx | cvx | cvx | cvx | cvx |
| Texture | mcfg | mcfg | mcsg | mcsg | mcsg | mcsg | mcsg | mcsg | mcsg |
| EPS production | md | md | cp | cp | cp | cp | cp | cp | dry |
| Colony shape | c | c | c | c | c | i | c | ndc | c |
| Nature of growth | cg | cg | cg | cg | cgflp | cg | cg | cg | cg |
| Percentage of the isolates | 4.24 | 4.24 | 3.39 | 7.63 | 4.24 | 31.36 | 42.37 | 0.85 | 1.69 |

e, entire; -ve, gram negative; cy, cream yellow; y, turned yma with btb yellow; crna, congo red non absorbing; mw, milky white; cw, cream white; mw+a, milky white in colour with grey rib like striations and milky white spot; cm+b, curdled milky with irregular tinny white spots in confluent colonies; ws, white, spotted in the middle ; w, watery; mw+o, milky white with opaque white centre; p, pink; o, opaque; t, translucent; cg, confluent growth with age; cgflp, confluent growth with flower like patterns; c, circular; i, irregular shape; ndc, no distinct colony; md, moderate; cp, copious; dry, no eps production; cvx, convex; colony ɵ, colony diameter; mcfg, mucoid firm gummy; mcsg, mucoid soft gummy

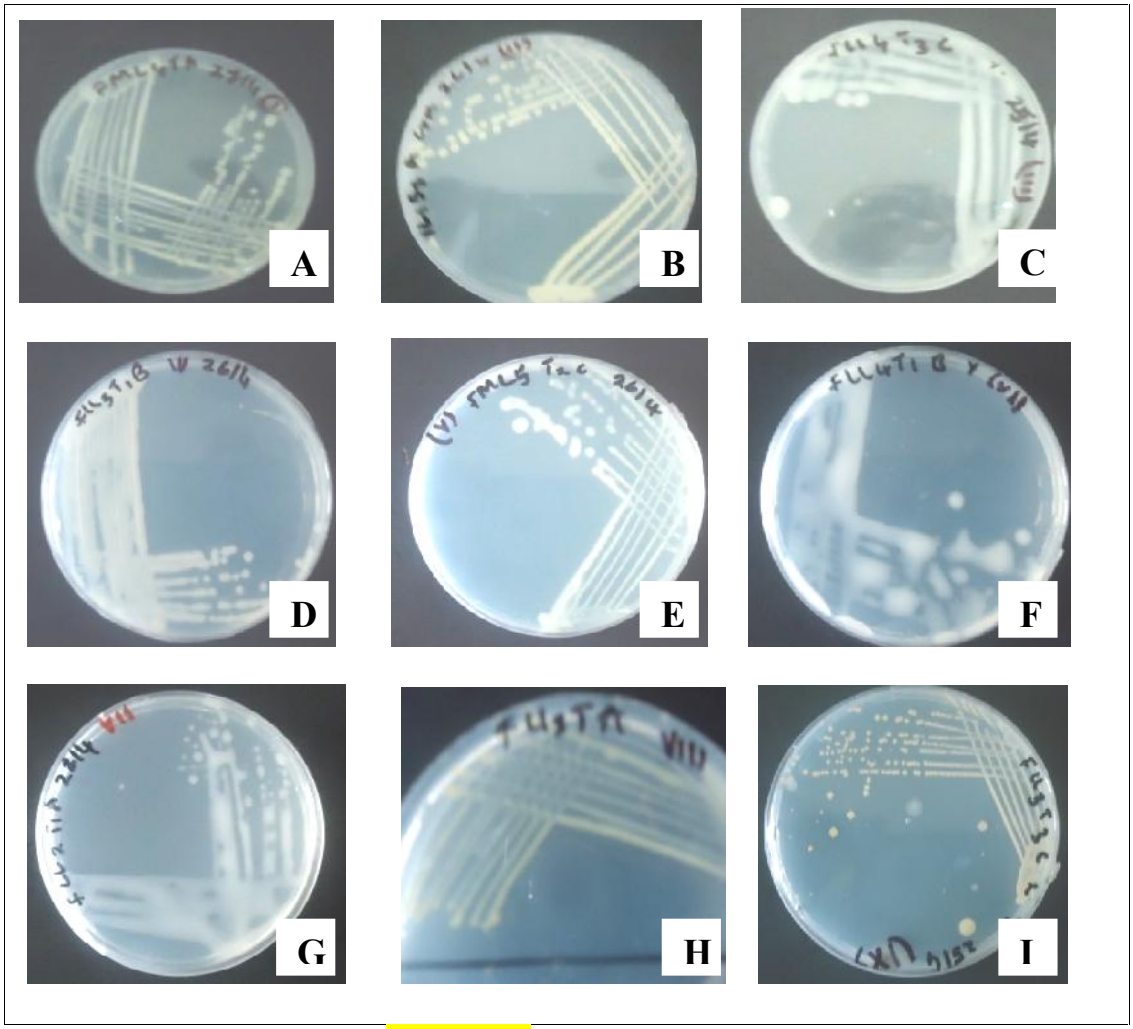


**Fig. 2.** Rhizobia isolates **from *P. vulgaris* nodules in whole soil trapping experiment. A, Group i; B, Group ii; C, Group iii; D, Group iv; E, Group v; F, Group vi; G, Group vii; H, Group viii; I, Group ix**

**Table 4. Rhizobia isolates obtained from the nodules of *P. vulgaris* plants grown in farms amended with water hyacinth compost in Korando B sulocation in Kisumu, Kenya**

| Isolate characteristics | Rhizobia isolate groups | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | i | ii | iii | iv | v | vi | vii | viii | x | xii | xiii | xiv | xv | xvii | ix |
| Margin | e | e | e | e | e | e | e | e | e | e | e | e | e | e | e |
| Gram reaction | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve |
| BTB reaction | y | y | y | y | y | y | y | y | y | y | y | y | y | y | y |
| Congo red absorption | crna+p | crna | crna | crna | crna | crna | crna | crna | crna | crna+p | crna | crna | crna | crna | crna |
| Color | pwc | cy | y | cws | cws | cw | mw | cw | c | mw | c | c+a | y | cy | c |
| Transparency | to | t | t | to | t | o | t | t | t | o | t | to | o | t | to |
| Cell shape | rod | rod | rod | rod | rod | rod | rod | rod | rod | oval | rod | rod | rod | rod | rod |
| Nature of the colony | shiny | shiny | shiny | shiny | shiny | shiny | shiny | shiny | shiny | shiny | shiny | shiny | shiny | shiny | shiny |
| Colony Ɵ (mm) | 4.1 | 0.5 | 2 | 3.75 | 0.5 | 1.9 | 1 | 1 | 2 | 7.25 | 2.4 | 5.3 | 2 | 2.4 | 1.3 |
| Elevation | d | cvx | cvx | cvx | cvx | cvx | cvx | cvx | d | cvx | cvx | cvx | cvx | cvx | rs |
| Texture | mcefg | mcsg | mcsg | mcsg | mcsg | mcfg | mcsg | mcsg | mcsg | mcfg | mcfg | mcsg | mcsg | mcfg | mcsg |
| EPS production | cp | md | md | cp | md | md | cp | cp | md | md | md | cp | cp | md | md |
| Colony shape | cir | cir | cir | cir | cir | cir | cir | cir | cir | cir | cir | cir | cir | cir | cir |
| Nature of growth | cg | cg | cg | cg | cg | cg | cg | cg | cg | cg | cg | cg | cg | cg | cg |

e, entire; -ve, gram negative; cp, copious; md, moderate; d, domed,; cvx, convex; rs, raised; mcefg, mucoid- elastic (firm gummy); mcsg, mucoid (soft gummy); mcfg, mucoid (firm gummy); cg, confluent growth; pwc, pinkish (white centre); cws, creamy ( with white suspension); cw, cream white; mw, milky white; c+a, creamy with opaque centre, confluent colonies with grey rib like striations; c, creamy; y, yellow; cy, cream yellow; to, transluscent (opaque center); t, transluscent; o, opaque; cir, circular; crna, congo red non absorbing; crna+p, congo red non absorbing with purple pigmentation on media; y, turns YMA media with BTB yellow, Colony; Colony diameter in mm

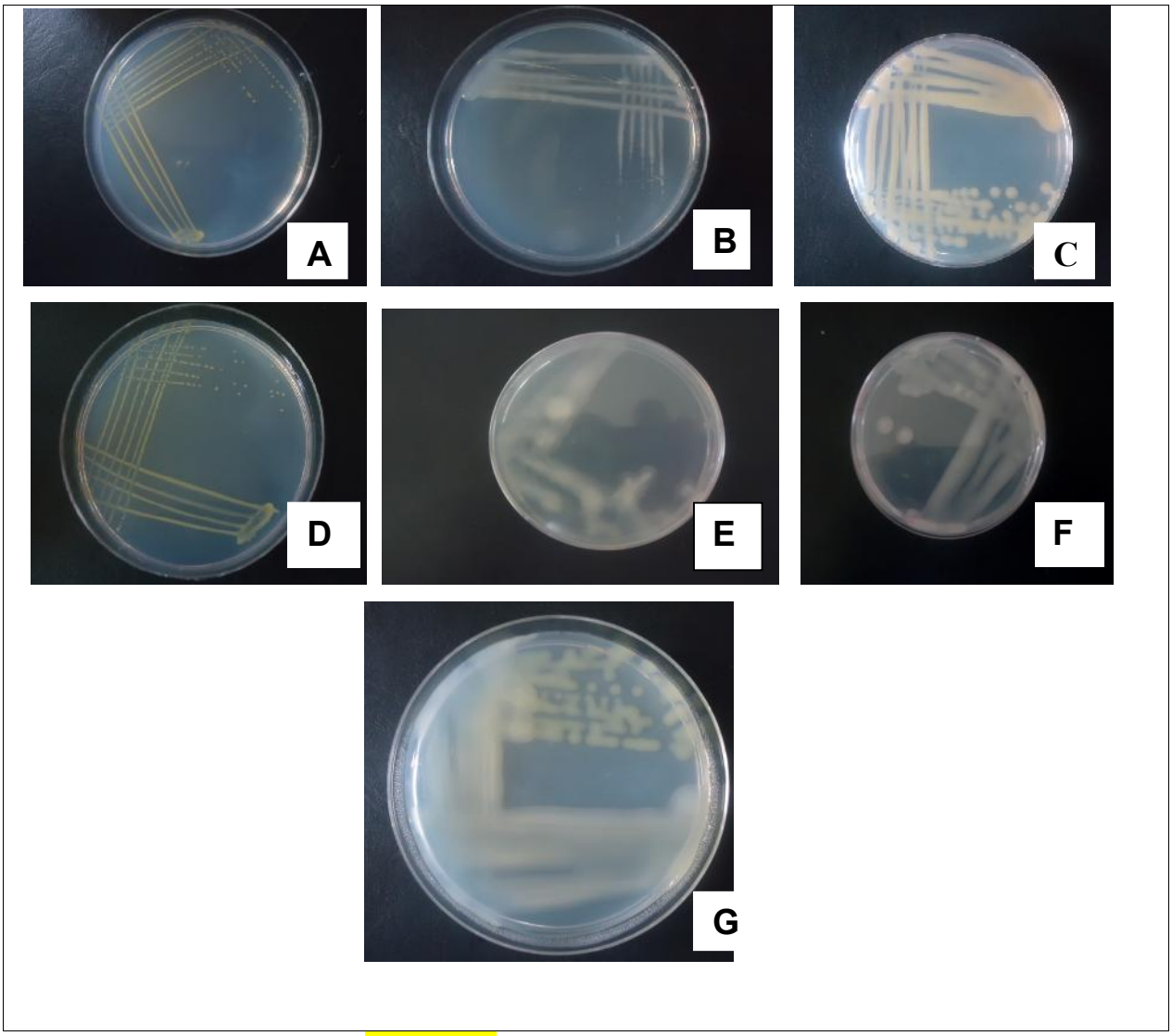


**Fig 3. Rhizobia isolates from *P. vulgaris* nodules from on farm trapping after soil amendment with water hyacinth compost. A, Group iii; B, Group v; C, Group xv; D, Group xvii; E, Group vii; F, Group viii; G, Group iv**

**Table 5. Abundance (%) of rhizobia isolates from nodules of *P. vulgaris* plants grown in water hyacinth compost testing farms in Korando B sub-location in Kisumu, Kenya**

| | Percentage number of isolates per farm | | | | |
|---|---|---|---|---|---|
| **Group** | **Farm A** | **Farm B** | **Farm C** | **Farm D** | **Authentication** |
| x | 12.9 | 6.59 | 4.1 | 0 | Infective |
| xiv | 4.03 | 3.59 | 1.64 | 4.23 | Infective |
| viii | 1.61 | 0.6 | 2.46 | 1.41 | Infective |
| xvi | 10.48 | 2.99 | 0 | 5.63 | Infective |
| iv | 39.52 | 32.33 | 37.7 | 28.17 | Infective |
| vii | 20.16 | 17.96 | 24.59 | 21.13 | Infective |
| xiii | 5.65 | 0 | 0 | 0 | Infective |
| vi | 4.03 | 3.59 | 4.91 | 8.45 | Infective |
| ix | 1.61 | 7.19 | 9.84 | 21.13 | Infective |
| iii | 0 | 13.77 | 3.28 | 8.45 | Infective |
| v | 0 | 0 | 0.82 | 1.41 | Infective |
| i | 0 | 5.39 | 0 | 0 | Infective |
| xii | 0 | 4.79 | 8.2 | 0 | Infective |
| xv | 0 | 0 | 1.64 | 0 | Infective |
| ii | 0 | 1.2 | 0 | 0 | Infective |
| xvii | 0 | 0 | 0.82 | 0 | Infective |
| **Total** | 100 | 100 | 100 | 100 | |

The cluster analyses demonstrated that there was only two main phenotypic groups for both whole soil trapping (Group I and II) and on farm trapping (Group A and B) rhizobia isolates as shown in Fig 4 and 5. Phenotypic cluster I of the whole soil trapping rhizobia isolates were the majority and had eight subgroups. Phenotypic cluster II of the whole soil trapping rhizobia isolates, represented minority of the isolates with only one subgroup. Phenotypic

cluster II showed a distant relationship from phenotypic cluster I of the whole soil trapping isolates. Phenotypic cluster A contained most of the on farm trapping isolates and had fourteen subgroups, with subgroups vii and viii, and ii and v, showing close relationships. Phenotypic cluster B with only one subgroup (xii) has a distant relationship from cluster A rhizobia isolates.

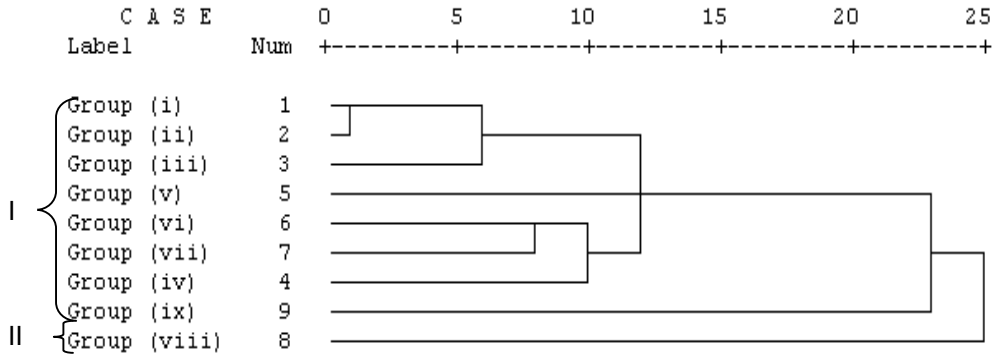


**Fig 4. Dendrogram showing morphological diversity of whole soil trapping rhizobia isolates from Lake Victoria Basin**

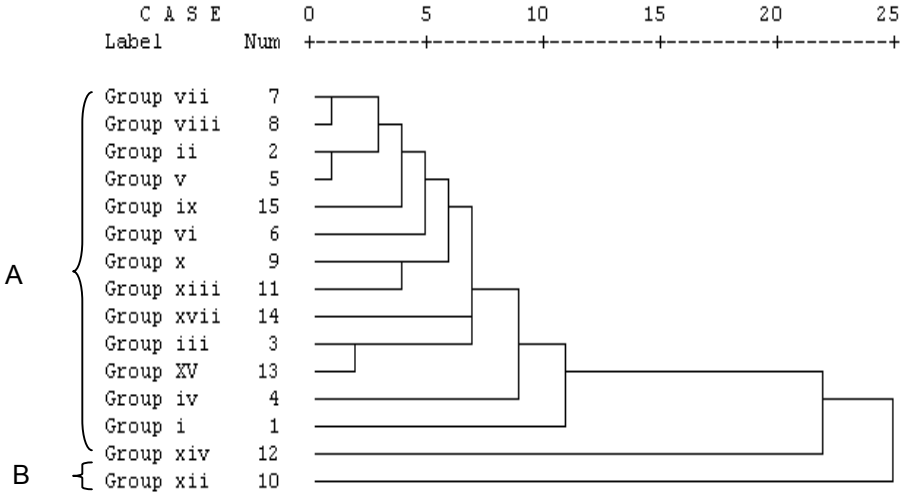


**Fig 5. Dendrogram showing morphological diversity of on farm trapping rhizobia isolates from Lake Victoria Basin**

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### 3.5 Authentication of rhizobia isolates

The isolates obtained in this study had colony characteristics of fast growing rhizobia and the majority of the tested isolates had the ability to re-nodulate *P. vulgaris* under bacteriologically controlled conditions. This is concordant with Bala et al. [38], who reported that appropriate rhizobia isolates nodulate and fix di-nitrogen on the target host and that each isolate that was able to form nodules with the host plant was identified as rhizobia. The results of nodulation ability (infectiveness) and plant dry matter response (effectiveness) of the isolates inoculated were variable as shown in Tables 6 and 7. This is concordant with the a previous study that have demonstrated that there is disparity in symbiotic effectiveness among indigenous rhizobia strains linked with particular host species [55].

There were significant differences in nodule number, nodule and shoot dry weights (P = 0.00) of *P. vulgaris* inoculated with rhizobia isolates obtained from whole soil trapping experiments (Table 6). There was no significant differences in root dry weight (P = 0.263). The mean nodulation ranged from 0.17 nodules to 192.00 nodules, showing the different ability of the rhizobia isolates to infect the host plant. Isolates FML2 S 1 II representing group II and FML6 2 CMX 1 representing group 1 from Korando B sites in Kisumu did not infect the host plant and therefore they were confirmed not be rhizobia, however isolate KIGALI 3 II from Rwanda with similar morphological characteristics as isolate FML2 S 1 II nodulated. This could be possibly due to the loss of the Kenyan isolate's viability during storage or to the probability of finding non-effective or deleterious rhizobial isolates isolated from the nodules [55]. There was no nodulation in the uninoculated control demonstrating that aseptic conditions were met in the experimental set up and maintenance of the plants in the greenhouse [38]. Commercial strain 446 had a lower infectivity potential with a mean nodulation of 0.67 nodules per plant as compared to some of the isolates like KIGALI 3 II that recorded mean nodulation of 192.00 nodules per plant. Nodulated plants had higher

shoot dry weight, than the non nodulated plants, however, the mean shoot dry weights was not directly related to the nodule number or nodule dry weight as observed in Table 6 and 7. This is in agreement with previous work, that has shown that nodule number and nodule dry weight is not appropriate for determining the effectiveness of a rhizobia – legume association [47, 56]. The significant differences in the shoot dry weights show clear differences in the ability of the isolates to fix nitrogen and are among the preferred methods for determining symbiotic effectiveness of rhizobia isolates [57].

The ability of the isolates to fix nitrogen was also demonstrated by observable differences in the plant colour and nodulation (Fig 6). The colour of the leaves of the plants depended on the effectiveness of the rhizobia isolate (Fig. 6). The leaves of plants inoculated with more effective isolates, had deep green color as opposed to the uninoculated control and plants inoculated with less effective isolates that were chlorotic with green yellow leaves. The dark green color observed in some of the inoculated treatments and not in the un-inoculated control showed effective symbiotic relationship between the common bean plant and some of the isolates after the sixth week of development. This corresponds well with previous works [56]. Strain 446 was poor in infectiveness and effectiveness as shown in Fig. 6G and Table 6.

There were significant differences in nodule number, nodule, root and shoot dry weights of the bean plants inoculated with isolates obtained after on farm trapping experiments ($p = 0.00$) (Table 7). The mean nodule number ranged from 0.67 for isolate (OW 1 D V) from farm D to 326.00 for isolate P9B614' from farm B. Bean plants inoculated with isolate P9B6 14 also had the highest shoot and root dry weight of 3.0 and 0.79 g respectively. Most of the bean crops inoculated with representative isolates from the LVB had higher mean shoot dry weight as compared to the locally available commercial standard strain 446 which had a mean shoot dry weight of 0.48 g (Table 7). The performance of strain 446 was consistent in the two authentication experiments as shown in Table 6 and 7. When isolate CP 5 VII that represents majority of the farm isolates was inoculated onto the bean plants the mean shoot

dry weight of 1.60 g was obtained (Table 7) and therefore performed better compared to the reference strain 446.

**Table 6. Infectiveness and effectiveness of representative isolates obtained from whole soil trapping experiments in the greenhouse**

| Isolate | Group | [1]Mean Nod no. | [2]NDW | [3]RDW | [4]SDW |
|---|---|---|---|---|---|
| FML 13 T 2 1 | I | 0.17a$^{+}$ | 0.00a$^{+}$ | 0.26a$^{+}$ | 0.32a$^{+}$ |
| FML2 S 1 II | II | 0.00a | 0.00a | 0.35a | 0.44a |
| FML3 S 2 X | X | 128.00ab | 0.16a | 0.28a | 1.21ab |
| FML 6 V | V | 45.33ab | 0.08abc | 0.36a | 0.95ab |
| FML 3 S I IX | IX | 67.50ab | 0.04abc | 0.33a | 0.83ab |
| FML6 2 CMX 1 | I | 0.00a | 0.00a | 0.33a | 0.65a |
| FLL3 T A 2ND IS VIII | VIII | 1.00a | 0.00a | 0.34a | 0.56a |
| FLL5 T1A IV | IV | 29.83a | 0.01ab | 0.38a | 0.69a |
| FLL 1 2 III | III | 124.50ab | 0.15abc | 0.24a | 1.47ab |
| FLL4 T3 B VII | VII | 87.83ab | 0.06abc | 0.29a | 0.90ab |
| FLL 1 Y | | 2.00a | 0.00ab | 0.47a | 0.78ab |
| FLL 3 T D VI | VI | 182.00b | 0.18cd | 0.30a | 1.44ab |
| VL5 S 1 C VI | VI | 89.67ab | 0.10abc | 034a | 1.46ab |
| U25TH ISOLATE III | III | 1.50a | 0.00ab | 0.28a | 0.71ab |
| U2 2$^{ND}$ ISOLATE IV | IV | 50.00ab | 0.05abc | 0.30a | 0.55a |
| UI I $^{ST}$ ISOLATE VII | VII | 117.00ab | 012abc | 0.19a | 1.25ab |
| U 2 V | V | 6.67a | 0.01ab | 0.37a | 0.46a |
| U2 5$^{TH}$ ISOLATE 111 | III | 10.33a | 0.00ab | 0.40a | 0.65a |
| KIGALI 3 II | II | 192.00b | 0.16abc | 0.30a | 1.66ab |
| RB2 VII | VII | 150.17ab | 0.16abc | 0.96a | 0.90ab |
| RB 3 CV | V | 181.00b | 0.20c | 0.25a | 2.15b |
| STRAIN 446 | Standard strain | 0.67a | 0.00a | 0.38a | 0.46a |
| Control | -ve control | 0.00a | 0.00a | 0.31a | 0.46a |

| | | | | |
|---|---|---|---|---|
| P. value | 0.000 | 0.000 | 0.263 | 0.000 |

[1]Nodule number, [2]Nodule dry weight, [3]Root dry weight, [4]Shoot dry weight.
[+]Values followed by the same letters within the columns are not significantly different from each other according to Tukey`s Honest Significant Difference (HSD) at 5 % level.

Isolates CP 18 A W, CP5 Vii, P10 Ok 4 (2), P 18 OK D4, OW 1 D14, P9 B 6 14' , FLL1 2 iii, FLL3TD Vi, VL5 S1C Vi, UI I$^{ST}$ ISOLATE Vii, Kigali 3 II, and RB3C v were more effective compared to the commercial strain 446 and are potential candidates for production of a more efficient and suitable inoculum for use in the Lake Victoria basin soils.

**Table 7: Infectiveness and effectiveness of representative rhizobia isolates obtained from on-farm trapping experiments**

| ISOLATE | Group | NOD NO.[1] | NDW[2](g) | SDW[3](g) | RDW[4](g) |
|---|---|---|---|---|---|
| Control | -ve control | 0.00a[+] | 0.00a[+] | 0.33a[+] | 0.38abc[+] |
| OW 1 D V | V | 0.67a | 0.00a | 0.47a | 0.32ab |
| P10 A II W | XII | 1.00a | 0.00a | 0.50ab | 0.42abc |
| NGT 14 A 3 | XIII | 1.00a | 0.00a | 0.40a | 0.26ab |
| P9OK VIII | VIII | 1.67a | 0.00a | 0.47a | 0.40abc |
| NGT 15 A 5 | XV | 11.00a | 0.00a | 0.53ab | 0.40abc |
| NGT 10 A 7 | X | 23.00ab | 0.00a | 0.57ab | 0.34ab |
| P10D III W | XII | 24.00ab | 0.00a | 0.77ab | 0.55bc |
| P4D Z | IX | 29.00ab | 0.00a | 0.67ab | 0.39abc |
| STRAIN 446 | Standard strain | 38.50ab | 0.02a | 0.48ab | 0.23ab |
| NGT 16B6 | VI | 53.67ab | 0.07a | 0.75ab | 0.39abc |
| P10 C III | III | 55.80ab | 0.06a | 0.52ab | 0.34ab |
| P17 OK MXD 17 | XVII | 57.60ab | 0.00a | 0.68ab | 0.29ab |
| P10 A (II)Y | II | 58.83ab | 0.02a | 0.80ab | 0.22ab |
| CP 18 A W | XII | 84.33ab | 0.07a | 1.07ab | 0.39abc |
| P 1 C (I) | I | 90.00ab | 0.00a | 0.50ab | 0.38ab |
| CP 5 VII | VII | 97.00ab | 0.10a | 1.60abc | 0.31ab |
| P13 OK 4 ( 2) | IV | 104.33ab | 0.07a | 1.10ab | 0.14a |
| P18 OK D 4 | IV | 203.67ac | 0.07a | 2.33bc | 0.60bc |
| OW 1 D 14 | XIV | 206.67bc | 0.17ab | 1.33abc | 0.38ab |
| P9B614' | XIV | 326.00c | 0.37b | 3.00c | 0.79c |
| P. value | | 0.00 | 0.00 | 0.00 | 0.00 |

[1]Nodule number, [2]Nodule dry weight, [3] Root dry weight, [4]Shoot dry weight.

[+]Values followed by the same letters within the columns are not significantly different from each other according to Tukey`s Honest Significant Difference (HSD) at 5 % level.

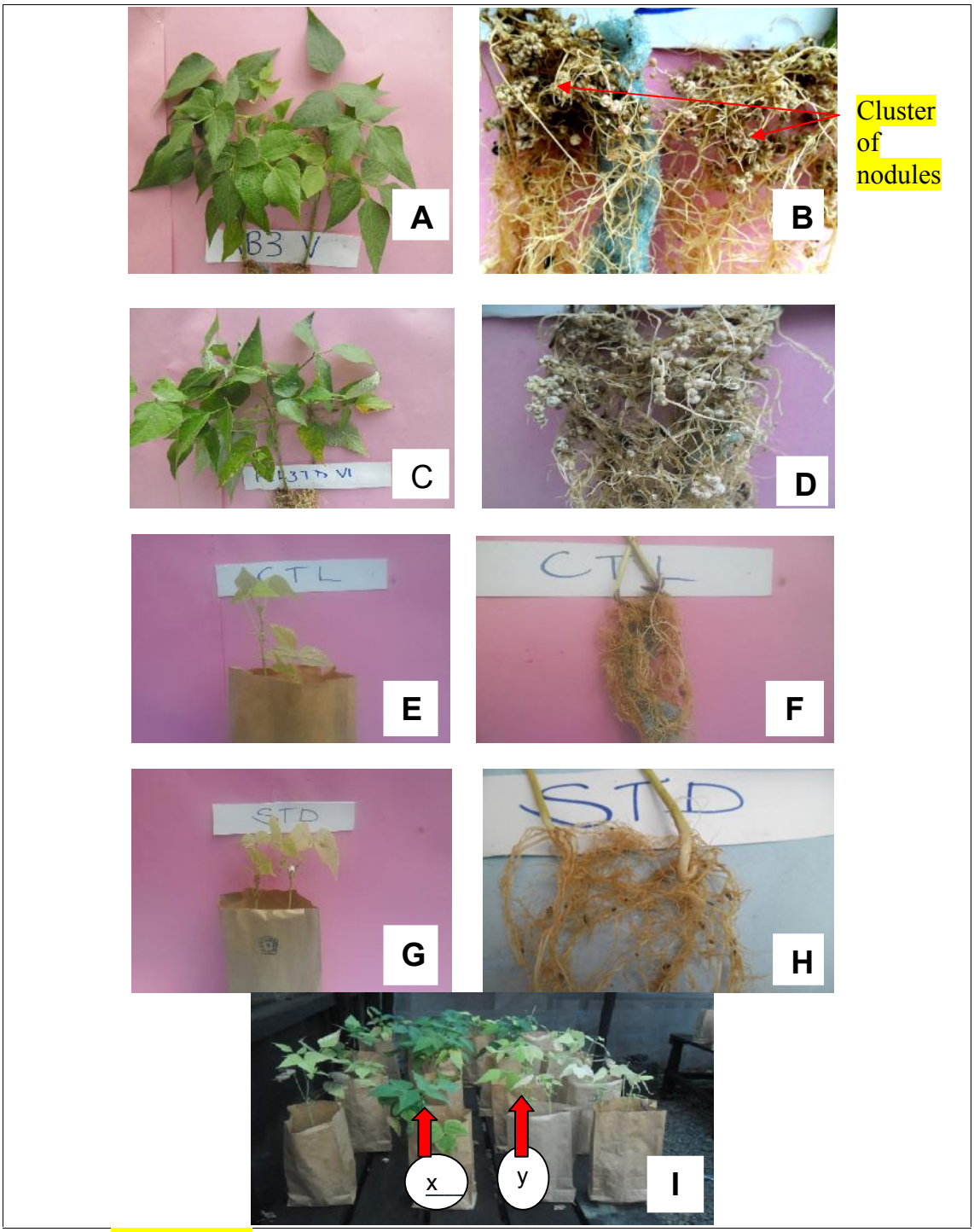


**Fig 6. A, *P. vulgaris* treated with isolate RB V (Group V) from Rwanda; B, Nodules formed as a result of isolate RB V from Rwanda; C, Effect of isolate FII3TD VI (group VI) from Kisumu, Kenya on *P. vulgaris*; D. Nodulation as a result of Isolate VI from Kenya; E, Un inoculated *P. vulgaris*; F, no nodulation of un inoculated *P. vulgaris*; G, *P. vulgaris* inoculated with standard strain 446; H, Poor nodulation; I, Effect of different rhizobia isolates on *P. vulgaris* ( x, effective strain, y, less effective strain)**

## 4. CONCLUSION

Results on the authentication experiments, confirmed that the majority of the isolates were rhizobia due to their ability to infect the host plant *P. vulgaris*. As demonstrated by the cluster analysis, there was high diversity of the isolates obtained from both whole soil trapping and onfarm trapping of *P. vulgaris* nodule isolates. Representative nodule isolates demonstrated varied ability to infect the host plant and fix nitrogen. Isolates that are more effective compared to the commercial strain 446 were identified in this study and will be tested further for possible innoculum production for Lake Victoria basin soils.


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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. Morris Muthini, John M. Maingi, John O. Muoma, Alice Amoding, Dative Mukaminega, Newton Osoro and Omwoyo Ombori collected, prepared the field samples and contributed in the experimental set up. Morris Muthini, Omwoyo Ombori and John M. Maingi handled the literature search and review, designed the study, performed the statistical analysis and drafted the first draft of the manuscript. Omwoyo Ombori and John M. Maingi read and approved the final manuscript.

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