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# Molecular Characterization of Rhizobia Nodulating Five Horse Gram [*Macrotyloma Uniflorum* (Lam.) Verdc.] Accessions Grown in a Greenhouse

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**ABSTRACT**
Grain legumes, such as Horse gram [*Macrotyloma uniflorum* (Lam.) Verdc.], form nitrogen-fixing symbiotic association with root nodules, however, information on horse gram-rhizobial association is still limited. This study characterized the Rhizobia associated with Horse gram accessions in Nigeria. The molecular characterization of true rhizobial isolates was done by 16S rRNA gene sequencing method. The study identified 21 true rhizobial strains to belong to genera *Rhizobium*, *Bradyrhizobium, Mesorhizobium* and *Ensifer,* with *Bradyrhizobium* strains as the most dominant genus. Therefore, isolation of efficient native nitrogen-fixing bacteria from horse gram plants could serve as bio-inoculants to improve legume production in Nigeria.

**Keywords:** Legumes, Rhizobia, *Macrotyloma Uniflorum*, Bio-Inoculants

## INTRODUCTION

Horse gram (*Macrotyloma uniflorum*), a leguminous crop belonging to the family *Fabaceae* and subfamily *Faboideae*, is an underutilized pulse and fodder crop native to Southeast Asia and tropical Africa (Bhartiya *et al*., 2015; Jha *et al*., 2022). Botanically, it is an annual herb growing to a height of 30–40 cm (Neelam *et al*., 2014). It is a drought-tolerant crop plant that grows successfully with low rainfall (Singh *et al*., 2017).

Nutritionally, horse gram is an excellent source of protein (17.9–25.3 %), carbohydrates (51.9–60.9 %), essential amino acids, energy, low lipid content (0.58–2.06 %), iron, molybdenum, phosphorus, and vitamins such as carotene, thiamine, riboflavin, niacin and vitamin C (Chahota *et al*., 2020). Horse gram grains have been reported to effectively contribute to a balanced diet and prevent widely diffused diseases, including type II diabetes and cardio vascular diseases (Prasad and Singh, 2015). Besides the nutritional and medical benefits of this legume, the symbiosis formed through its interaction with rhizobia can transform atmospheric nitrogen to ammonia by supplying nitrogen to the plant and enhancing its ability to withstand nickel stress even in arid environments (Edulamudi *et al*., 2021). By reducing the need for synthetic nitrogen fertilizers, the interaction contributes to sustainable agriculture through enhancement of soil fertility and crop productivity and thus, it mitigates environmental impact of nitrogen runoff in water bodies and the emission of nitrous oxide, a potent greenhouse gas (Dent and Cocking, 2017; Bogale *et al*., 2023). However, the characterization of the rhizobia could be important for the development of effective inoculants and the improvement of the crop's nitrogen fixation ability (Amaral *et al*., 2022). This study was therefore carried out to assess genetic diversity of rhizobial strains that could profusely nodulate horse gram (*Macrotyloma uniflorum*) with the aim of developing the effective microbial inoculants for sustainable production of horse gram in Africa.

## MATERIALS AND METHODS

Five different accessions (TMu1, TMu2, TMu4, TMu6 and TMu8) of horse gram seeds (Plate 1), sourced from the Genetic Resources Center of the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, were used in this study. The seeds were surface-sterilized, air dried and sown in pots filled with loamy sand soil (68% sand, 18% silt, 14% clay and pH 6.0). The pot experiment, which was performed in the greenhouse of Pure and Applied Botany, College of Biosciences, Federal University of Agriculture, Abeokuta, consisted of five accessions of horse gram and three replications per accession arranged in a completely randomized design. Each pot was sown with two seeds and thinned to one plant per pot at 7 days after sowing (DAS). The experiment lasted for 45 days with regular watering with no application of fertilizer.



Plate 1: Seeds of Horse Gram Accessions used

At 45 days after sowing, the horse gram plants were carefully uprooted and washed off the root without detaching the nodules. The nodules were carefully detached from the roots, counted and weighed. Five red or pink, large, healthy and unbroken nodules were selected and used for nodule bacterial isolation using the culture-dependent method described by Oloyede *et al*. (2022) with slight modifications. The number of rhizobial colonies on yeast extract mannitol agar plates were counted and recorded as total population of nodule rhizobia. The pure rhizobial isolates were obtained and further authenticated on their respective host plants using plant nodulation test as described by Oloyede *et al.* (2024) with slight modifications. The isolates that formed effective nodulation on the test host plants were considered as true rhizobia. The rhizobial isolates were characterized by phenotypic (colonial, morphological and biochemical) and molecular methods.

The molecular characterization of the rhizobial isolates was performed using 16S rRNA gene sequencing technique. Isolation of DNA was done using zymo quick-DNA fungal/bacterial miniprep kit (Zymo research catalogue No.D6005), followed PCR amplification of 16S rRNA genes of the isolates using the universal primers for bacterial amplification, 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) as forward and 1492R (5′-ACG GCT ACC TTG TTA CGA CTT-3') as reverse. The PCR products were purified with Zymo Research, ZR-96 DNA sequencing clean- up kit and sequenced in ABI 3500XL Genetic analyzer. The sequences were then assembled, aligned and identified using the BLASTn program of GenBank database of National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov.).

Analysis of data collected was done using one-way analysis of variance test of Statistical Package for Social Sciences (SPSS) version 23.0 for Windows (SPSS, Chicago IL, U.S.A), followed by separation of means by New Duncan's Multiple Range Test at $p \leq 0.05$.

## RESULTS AND DISCUSSION

The results of the study showed no significant ($p \leq 0.05$) difference in the number of nodules of five accessions of horse gram at 45 days after sowing (DAS), however, significant differences were observed in the fresh nodule weights. Accession TMu6 had the highest number nodules (22.67), closely followed by TMu1 while the least was found in TMu2 (18.33). However, TMu1 had a significantly ($p \leq 0.05$) higher fresh nodule weight (Table 1).

Table 2 presents the populations of rhizobia in the root nodules of five accessions of horse gram plants at 45 DAS. Though no significant ($p \leq 0.05$) difference was found in the rhizobial populations, highest population was obtained in accession TMu4 ($4.67 \times 10^3$ cfu/g of nodule) while the least population was found in TMu6 ($3.33 \times 10^3$ cfu/g of nodule).

Though 32 strains of Rhizobia were recovered from the root nodules of five accessions of horse gram seedlings at 45 DAS, only 21 strains (65.6%) were regarded as true rhizobia, being able to effectively nodulate their host plants. Previous studies by Ngo *et al.* (2015) and Oloyede *et al.* (2024) also noted that 66.7% and 42.9% of the bacteria nodulating the roots of bambara groundnut (another underutilized legume) were true rhizobia. Phenotypically, the colonies were mostly medium-sized, translucent, convex, white, raised and mucoid in texture. The strains were Gram negative bacteria, rod-shaped, motile and catalase positive. None of the strains was indole positive and most of them were able to ferment glucose, fructose and sucrose. The phenotypic characteristics exhibited by these bacterial strains are typical features of rhizobia nodulating leguminous plants. These results are in agreement with the findings of Datta *et al*. (2015), Oloyede *et al*. (2022) and Oloyede *et al*. (2024) who observed these characteristics in micro-symbionts nodulating the roots of clover, mung beans, soya beans, winged beans and bambara groundnut plants. Six of the true rhizobial strains were isolated from the root nodules of accession TMu 4, four strains from each of TMu 1, TMu 2 and TMu 8, while the remaining three strains were obtained from accession TMu6. Furthermore, the phenotypic and molecular characterization identified the strains to belong to four genera, *Rhizobium*, *Bradyrhizobium, Mesorhizobium* and *Ensifer,* with *Bradyrhizobium* strains as the most dominant genus (Table 3). These bacterial strains could be considered as horse gram rhizobia. This result correlates with the studies of Appunu *et al*. (2011) and Edulamudi *et al*. (2015) who revealed that the rhizobia nodulating horse gram belong to different rhizobial genera.

## CONCLUSION

The rhizobial strains associated with effective nodulation of landraces of horse gram in Nigeria are diverse, and include the species of *Rhizobium*, *Bradyrhizobium, Mesorhizobium* and *Ensifer,* Utilization of these strains as effective bioinoculants could enhance the cultivation of horse gram in poor soils, and this is likely to contribute to food security, reduces hungers and improves the livelihoods of many farmers in rural areas to achieve the Sustainable Development Goal 2, which is termed "End hunger, achieve food security and improved nutrition and promote sustainable agriculture.

**Table 1: Nodulation Potential of Different Accessions of Horse gram Seedlings at 45 Days after Sowing**

| Accessions | Nodule number/plant ± S.D. | Fresh nodule weight (g/ plant ± S.D.) |
|---|---|---|
| TMu 1 | $20.67 \pm 6.43^{a}$ | $1.80 \pm 0.26^{a}$ |
| TMu 2 | $18.33 \pm 2.31^{a}$ | $1.20 \pm 0.40^{bc}$ |
| TMu 4 | $19.67 \pm 4.040^{a}$ | $1.10 \pm 0.26^{c}$ |
| TMu 6 | $22.67 \pm 4.04^{a}$ | $1.70 \pm 0.26^{ab}$ |
| TMu 8 | $18.67 \pm 2.52^{a}$ | $1.50 \pm 0.26^{abc}$ |

Values are means of three plants per accession ± standard deviations. Means with the different letters along the columns are significantly different ($p \leq 0.05$) using New Duncan's Multiple Range Test

**Table 2: Rhizobial Populations of Root Nodules of Different Accessions of Horse gram Seedlings 45 Days after Sowing**

| Accessions | Total Rhizobial Counts (× $10^3$ cfu/g ± S.D.) |
|---|---|
| TMu 1 | 4.33 ± 1.53[a] |
| TMu 2 | 3.67± 0.58[a] |
| TMu 4 | 4.67 ± 1.53[a] |
| TMu 6 | 3.33 ± 2.08[a] |
| TMu 8 | 3.67 ± 1.53[a] |

Values are means of three plates per accession ± standard deviations. Means with the different letters along the columns are significantly different ($p \leq 0.05$) using New Duncan's Multiple Range Test

**Table 3: Phenotypic and Molecular Characterization of Rhizobia Nodulating Different Accessions of Horse Gram [*Macrotyloma uniflorum* (Lam.) Verdc.]**

| Isolate | Accession | Phenotypic identification | Molecular identification | Approx.% Similarity |
|---|---|---|---|---|
| HOG1 | TMu1 | *Rhizobium* spp. | *Rhizobium etli* | 98.0 |
| HOG2 | TMu1 | *Rhizobium* spp. | *Rhizobium leguminosarum* biovar *trifolii* | 95.0 |
| HOG3 | TMu1 | *Bradyrhizobium* spp. | *Bradyrhizobium japonicum* | 95.0 |
| HOG6 | TMu1 | *Mesorhizobium* spp. | *Mesorhizobium thiogangeticum* | 90.0 |
| HOG7 | TMu2 | *Bradyrhizobium* spp. | *Bradyrhizobium* spp. strain B918 | 98.0 |
| HOG8 | TMu2 | *Bradyrhizobium* spp. | *Bradyrhizobium japonicum* | 95.0 |
| HOG9 | TMu2 | *Mesorhizobium* spp. | *Mesorhizobium thiogangeticum* | 88.0 |
| HOG12 | TMu2 | *Rhizobium* spp. | *Rhizobium leguminosarum* biovar *trifolii* | 95.0 |
| HOG13 | TMu4 | *Rhizobium* spp | *Rhizobium etli* | 96.0 |
| HOG14 | TMu4 | *Bradyrhizobium* spp | *Ensifer meliloti* strain T1607 | 90.0 |
| HOG15 | TMu4 | *Rhizobium* spp. | *Rhizobium leguminosarum* | 90.0 |
| HOG17 | TMu4 | *Bradyrhizobium* spp. | *Bradyrhizobium japonicum* | 96.0 |
| HOG18 | TMu4 | *Bradyrhizobium* spp. | *Bradyrhizobium japonicum* | 99.0 |
| HOG20 | TMu4 | *Mesorhizobium* spp. | *Mesorhizobium* spp. strain SW3 | 95.0 |
| HOG21 | TMu6 | *Rhizobium* spp. | *Rhizobium leguminosarum* biovar *trifolii* | 98.0 |
| HOG23 | TMu6 | *Bradyrhizobium* spp. | *Ensifer* spp. strain LEM551 | 95.0 |
| HOG24 | TMu6 | *Bradyrhizobium* spp. | *Bradyrhizobium japonicum* | 98.0 |
| HOG25 | TMu8 | *Bradyrhizobium* spp. | *Bradyrhizobium japonicum* | 95.0 |
| HOG26 | TMu8 | *Bradyrhizobium* spp. | *Bradyrhizobium japonicum* | 95.0 |
| HOG30 | TMu8 | *Bradyrhizobium* spp. | *Bradyrhizobium japonicum* | 95.0 |
| HOG32 | TMu8 | *Rhizobium* spp. | *Rhizobium leguminosarum* biovar *trifolii* | 98.0 |

## REFERENCES


Amaral, M. B., dos Santos Lopes, T., Feder, C. B., Ribeiro, T. G., Pacheco, R. S., Teixeira, T. N., Monteiro, E. C., Ramalho, I. O., Macedo, R. O., Boddey, R. M., Zilli, J. E. and Alves, B. J. (2022). *Bradyrhizobium* occurrence in nodules of perennial Horse gram. *Brazilian Journal of Microbiology*. 53(4)**:** 2065 - 2075.

Appunu, C., Ganesan, G., Kalita, M., Kaushik, R., Saranya, B., Prabavathy, V. R., and Sudha, N. (2011). Phylogenetic diversity of rhizobia associated with Horse gram [*Macrotyloma uniflorum* (Lam.) Verdc.] grown in South India based on *glnII, recA* and *16S-23S* intergenic sequence analyses. *Current Microbiology.* 62**:** 1230-1238.

Bhartiya, A., Aditya, J. P. and Kant, L. (2015). Nutritional and remedial potential of an underutilized food legume horse gram (*Macrotyloma uniflorum*): a review. *Journal of Animal and Plant Sciences*. 25(4): 908 – 920.

Bogale, A. A., Melash, A. A. and Percze, A. (2023). Symbiotic and asymmetric causality of the soil tillage system and biochar application on soil carbon sequestration and crop production. *Soil Systems***.** 7(2)**:** 48. doi:10.3390/soilsystems7020048.

Chahota, R. K., Thakur, N. and Sharma, R. (2020). Efficient improvement in an Orphan legume: Horsegram, *Macrotyloma uniflorum* (Lam.) Verdc, using conventional and molecular approaches. In: Gosal, S. S. and Wani, S.H. (eds). Accelerated Plant Breeding. Volume 3: Food Legumes: Springer. 369-388.

Datta, A., Sing, R. and Tabassum, S. (2015). Isolation, characterization and growth of *Rhizobium* strains under optimum condition for effective biofertilizer production. *International Journal of Pharmaceutical Sciences Review and Research*. 32 (1)**:** 199-208.

Dent, D. and Cocking, E. (2017). Establishing symbiotic nitrogen fixation in cereals and other non-legume crops: The greener nitrogen revolution. *Agriculture and Food Security*. 6(7)**:** 1 - 9.

Edulamudi, P., Masilamani, A. J., Vanga, U. R., Divi, V. R. S. G. and Konada, V. M. (2021). Nickel tolerance and biosorption potential of rhizobia associated with horse gram [*Macrotyloma uniflorum* (Lam.) Verdc.]. *International Journal of Phytoremediation*, 23(11)**:** 1184 - 1190.

Jha, U. C., Nayyar, H., Parida, S. K. and Siddique, K. 2022. Horse gram, an underutilized climate-resilient legume: Breeding and genomic approach for improving future genetic gain. In: Developing climate resilient grain and forage legumes edited by Jha, U. C., Nayyar, H., Agrawal, S. K. and Siddique, K. H. M. Springer Link.Pp.167 – 178.

Neelam, S., Kumar, V., Natarajan, S., Venkateshwaran, K., and Pandravada, S. R. (2014). Evaluation and diversity observed in Horse gram (*Macrotyloma uniflorum* (Lam) Verdc.) germplasm from Andhra Pradesh, India. *International Journal of Plant Research*. 4(1)**:** 17-22.

Oloyede, A. R., Oyelakin, O. O. and Ogunesan, S. O. (2022). Phenotypic and Phylogenetic Characterization of Micro-symbionts Nodulating Winged Bean (*Psophocarpus tetragonolobus* L. DC.) Landraces. *West African Journal of Applied Ecology*. 30(1): 58 – 67.

Oloyede, A. R., Ojo, A. E. and Taiwo, E. A. (2024): Genetic Characterisation and Symbiotic Efficiency of Rhizobia Nodulating Different Landraces of Bambara Groundnut (*Vigna subterranean* L. Verdc.). *Nigerian Journal of Microbiology*. 38(1): 6987 – 6997.

Ngo, N. L., Ngo, B. M., Fankem H., Adamou, S., Kamguia, K., Ngakou, A., Nwaga, D. and Etoa, F. (2015). Isolation and screening of indigenous Bambara groundnut (*Vigna Subterranea*) nodulating bacteria for their tolerance to some environmental stresses. *American Journal of Microbiological Research.* 3 (2): 65 – 75.

Prasad, S. K., and Singh, M. K. (2015). Horse gram-an underutilized nutraceutical pulse crop: a review. *Journal of Food Science and Technology.* 52**:** 2489-2499.

Singh, P., Soni, P., Agrawal, J. and Tiwari, V. (2017). *Macrotyloma* is a blessed pulse for Satna district in Madhya Pradesh India. *International Journal of Applied Research and Technology.* 2(1)**:** 28 - 35.

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