EJSIT
European Journal of Science, Innovation and Technology
ISSN: 2786-4936
www.ejsit-journal.com
Volume 3 | Number 6 | 2023

Towards the Analysis of the Genetic Diversity of Dolichos Lablab (*Lablab Purpureus* (L.) Sweet) and Identification of Its Rhizosphere Bacteria in Namibia

Faith Fransisca Kavishe[1*], Jeya Kennedy[1], Percy Chimwamurombe[1], Jean Damascène Uzabakiriho[2]
[1]Department of Biology, Physics and Chemistry, Namibia University of Science and Technology, Namibia
[2]Department of Biological Sciences, University of Namibia, Namibia

ABSTRACT

Dolichos lablab (*Lablab purpureus* (L.) Sweet)) is a multipurpose drought tolerant protein-rich legume crop native to Africa and grown in warm temperate to tropical climates for its edible seeds and manure. *Lablab purpureus* holds significant benefits to subsistence farmers and offers a great promise for sustainable crop productivity, especially in marginalised areas. Its uses range from human consumption as a vegetable to improving soil fertility, and as forage. Notwithstanding *Lablab purpureus* crucial potential functions in Namibia, there is currently limited information regarding the plant's genetic diversity and its rhizospheric bacteria. Assessing the genetic similarity of these varieties through microsatellite analysis will significantly enhance the identification of distinctive ones for subsequent introduction. Future projections show that by 2050 the agricultural products demand for the market will increase by 70% and this will cause challenges for contemporary agriculture. Agricultural practices that make improper use of expensive, and environmentally harmful chemical pesticides and fertilisers are all issues that need to be addressed. Alternative ways of sustainably meeting agricultural demands involve using rhizobacteria or other microbial inoculants for plant growth and development. Understanding the composition of rhizosphere bacteria associated with these plants offers an avenue for discerning their potential contributions to enhancing soil fertility, facilitating nutrient cycling, mitigating disease prevalence, and fostering plant growth.

Keywords: Genetic diversity, *Lablab purpureus*, SSR, Microsatellite markers, 16s rRNA, rhizosphere, rhizosphere bacteria, Namibia

INTRODUCTION TO *LABLAB PURPUREUS*

Dolichos lablab [*Lablab purpureus (L)* Sweet] also known as Indian bean, hyacinth bean, bonavist bean, and field bean (Sheahan, 2012). It is an underutilised (orphan) drought and heat tolerant crop from the family *Fabaceae* grown in America, Asia, and Africa in the semi-arid and dry regions for use as feed and food (Minde et al., 2020). This legume is a semi erect perennial herb that is mostly self-pollinating with doubled chromosome number of 2n=2x=22 (Sserumaga et al., 2021).

Lablab purpureus is typically an herbaceous perennial, however, it is grown as an annual or biennial, and has a climbing, bushy, or dwarf habit. Its stem is 2-3 m long, but it can reach 10 m in length (Chamarthi et al., 2011). Figure 1 shows pictures of some species of *Lablab purpureus*. It is particularly suited to dry environments and is sown on riverbanks following a reduction in water levels. It is usually on the flat when grown for pulses or stock feed or is supported on canes or other structures when cultivated as a vegetable (Chamarthi et al., 2011).

* Corresponding Author

Figure 1: Leaves, flowers, and fruits of various species of *Lablab purpureus* (Moteetee & Van Wyk, 2012)

The adaptability of this species is quite extensive, being able to tolerate summer mean temperature in the range of 22˚– 35˚C, altitudes up to 2100m, acidic to alkaline varieties of soils and annual precipitation from 200- 2100mm (Wang et al., 2016). When compared to other legumes it has a higher adaptation to drought conditions, making it a highly drought tolerant legume (Raghu et al., 2018). Wang et al. (2016) describe two botanical types of *Lablab purpureus*, the bushy and erect field type and a garden type characterised with twining branches and stems.

Lablab purpureus is endowed with several benefits; young, seeds and green pods are eaten as a low lipid and carbohydrate delectable vegetable, and extraordinarily rich source of dietary fibre. Once matured, the harvested dry seeds are kept and consumed as a pulse throughout the year (Raghu et al., 2018). Nutritionally, it is a useful source of protein, minerals (Sulphur, Magnesium, Phosphorus, Sodium, Iron and Calcium), vitamins (Riboflavin, C and A), and amino acids (lysine) (Raghu et al., 2018). According to Wang et al. (2016) seeds and leaves of *Lablab purpureus* contain 20-28% protein. This makes it nutritionally important for healthier food habits as low fat and low-calorie vegetarian foods are gaining popularity with changing lifestyle patterns (Raghu et al., 2018). It is used as either a pulse or for the young pods which are used for stock feed or as a fresh vegetable. The field type is employed as a cover crop, green manure, forage, and to produce seeds, whereas the garden type is used mostly as a green vegetable.

In the South Indian diet, this legume serves as the main protein source. It is also popularly grown in the northeast and eastern parts of the country. This crop is either intercropped with groundnut and other cereals like sorghum, corn, pearl millet and finger millet or grown in pure stands (Raghu et al., 2018). The green pods, fresh leaves, mature grains, and immature grains are used for some medicinal purposes (Murphy & Colucci, 1999).

In animal feeding, it is used as fresh forage, grain, straw, grazing, hay, browsing or forage meal (Murphy & Colucci, 1999). The great crude protein of *Lablab purpureus'* green leaves adds to the nutritional content and palatability of green fodder. It is similarly used when making better quality silage with improved protein content. The better-quality silage is obtained by mixing it with other cereals such as sorghum in a 2:1 ratio (Raghu et al., 2018).

This crop is useful in weed control, soil protection and improvement. It is a good cover crop, especially in dry seasons when its dense green cover reduces the effects of wind or rain erosion and protects the soil from harsh rays of the sun (Murphy & Colucci, 1999). The plant

adds more organic carbon to soil and fixes atmospheric nitrogen symbiotically with beneficial microorganisms, thus improving the fertility of soil and the wide root system it has, aides in improving both the structural and physical characteristics of soil (Raghu et al., 2018).

This review aims at assessing the genetic diversity of *Lablab purpureus* varieties grown in Namibia using microsatellites (SSR) as the molecular markers.

GENETIC DIVERSITY

Genetic diversity is an assortment of genes, present in each species, that are essential for maintaining and adapting desired traits and ensuring survival during natural selection (McBenedict et al., 2016). Variations occur either through dissimilarities in DNA sequences, the amount of DNA found in cells and structure and number of chromosomes. Although it can be applied to different populations that are linked to the same environmental conditions, it is not limited to individuals within a population (McBenedict et al., 2016). It is a measure of biodiversity.

If a sizable portion of the genes in a population are polymorphic, that population is said to be genetically diverse. A polymorphic gene is one for which the frequency of the most prevalent allele is less than 0.95 (Jaskulak et al., 2022). Allele frequency is the proportion of an allele at a genetic locus that is present in a population (Rezaei & Hedayat, 2013). It is usually expressed as a decimal, percentage, or ratio. The formula for calculating it is by dividing the total number of alleles in the population at a specific locus by the frequency of a specific allele (Rezaei & Hedayat, 2013). The proportion of genes that are heterozygous and/or polymorphic serves as a measure of genetic diversity. Evolutionary or selective forces maintain, create, and enhance the heritable variation within populations (Rai et al., 2010).

TYPES OF MARKERS

Markers can either be morphological, biochemical, cytological, or molecular. Morphology requires visual identification to differentiate between different population species. Such visuals may be colours of flowers, colours of seeds, leaf shapes or textures. Such markers are usually influenced by environmental factors, so plants of the same species may appear differently if their geographic location is different (Antonio et al., 2017). Biochemical markers depend on protein binding patterns i.e., isozymes, to identify or differentiate between different species and these are visualised on protein gels. Cytological markers look at variations in chromosome number, size, shape, and binding patterns and are mostly used to identify genetic diseases and banding patterns of chromosomes (Gibson & Spencer, 2009). For molecular markers, a known DNA sequence (a gene) on a chromosome is used to identify individuals or species. Molecular markers may show multiple variants (alleles) at a specific locus on homologous chromosomes (Gibson & Spencer, 2009).

Molecular Markers

These are described as specific DNA segments that can be found throughout the entire genome at a particular location. Individuals' genetic variations are recognized using molecular markers (Gibson & Spencer, 2009).

Polymorphisms are abundant in every genome. Sequence sites that have two or more variants are known as polymorphic (Gibson & Spencer, 2009). A general assumption is that the heritable element of character variation i.e., quantitative genetic variation which include but are not limited to characters such as yield, size, disease susceptibility and shape should be traceable to insertion/ deletion polymorphisms or to Single Nucleotide Polymorphisms (SNPs) (Deschamps et al., 2012). The first and most important step in identifying the connections between phenotypic and SNP variation is characterising the distribution of SNPs. Variation in repetitive sequences (microsatellites) and in SNPs are crucial tools for determining

relationships between individuals, forensic investigations, evolutionary studies of a species' origins, and population structure studies (Gibson & Spencer, 2009).

The goal of molecular methods is to find naturally occurring polymorphisms at the DNA level. They have several advantages over morphological markers. They have high polymorphisms, the effects of various environmental factors have no influence on them, aren't tissue specific so any stage of an organism's development can yield evidence of them, and they aren't influenced by the physiological state of an individual. Very small amounts of the sample are sufficient for molecular analysis, and the physical forms of the sample have no bearing on DNA detection (Dholakia et al., 2019).

Classification of Molecular Markers

The different types of markers that are available can be categorised into various types of groups according to chronology or whether they use PCR or not. Chronologically there are the 1st generation markers such as Random Fragment Length Polymorphisms (RFLPs) which are not PCR based but rather use restriction endonucleases and hybridization techniques (Antonio et al., 2017). Microsatellites are of the 2nd generation, whereas 3rd generation markers include SNPs. The same authors report that PCR based markers can be dominant or codominant. The dominant markers only consider whether an allele is present or absent at a specific locus and can be denoted by; present (+ or 1) or absent (- or 0) respectively as viewed as bands on gel electrophoresis. An example of a dominant marker is Random Amplified Polymorphic DNA (RAPD). Co-dominant markers investigate the absence/ the presence of specific bands on an agarose gel and take into consideration the differential expression levels of that specific trait. The expression levels can be seen as either very high (seen as very bright bands), high, medium, or low (faint bands) (Antonio et al., 2017).

Molecular markers have found various applications such as in population studies, wildlife, forensics, eugenics, quantitative trait loci (QTL) analysis, genetic diversity studies, genetic linkage mapping to study genetic diseases, DNA barcoding and molecular identification (Gyang et al., 2017).

Molecular markers' variations arise due to changes in base pairs such as mutations, translocation or inversions, insertions or deletions, or Variable Number Tandem Repeats (VNTRs) or Short Tandem Repeats (STRs) (Gibson & Spencer, 2009). Good molecular markers are present uniformly throughout the genome (ubiquitous), are highly reproducible, should have a rapid and simple assay, should be stably inherited, shouldn't interact with other markers while using multiple markers at the same time and should be polymorphic in nature (Gyang et al., 2017). Polymorphisms, if interspecific, can distinguish between 2 different species. Within a single species, intragenic can demonstrate genetic diversity.

Characterisation of the germplasm of neglected orphan crops ensures their genetic diversity is preserved efficiently. Several tools are available for genetic assessments such as Inter simple sequence repeat (ISSR) markers, simple sequence repeat (SSR) markers which are known as microsatellites, amplified fragment length polymorphisms (AFLPs), and random amplified polymorphic DNA (RAPD) markers (Zhang & Lijuan, 2013). The SSR markers are highly suitable because they have polymorphic genetic information and are highly reproducible requiring only small amounts of template DNA (Kamotho et al., 2016; Keerthi et al., 2018).

MICROSATELLITES (SSRS)

As described by Shivachi et al. (2012) they are simple sequence repeats (SSRs) with up to 13 bases in each repeat. There is at least one dinucleotide, trinucleotide, and tetranucleotide repeat every 10 kb in the genomes of a variety of eukaryotic organisms. Microsatellites may result from quite a few mechanisms such as transposon- associated microduplications, replication slippage and some arise by substitution mutations (Gibson & Spencer, 2009). They

mutate much more frequently than conventional sequences, they have a high probability of back mutation and are very useful for figuring out the evolutionary connections between populations within a species; a mutation rate of up to 0.001 gametes per generation (Gyang et al., 2017). Since they evolve too rapidly, they aren't phylogenetically informative between species (Gibson & Spencer, 2009).

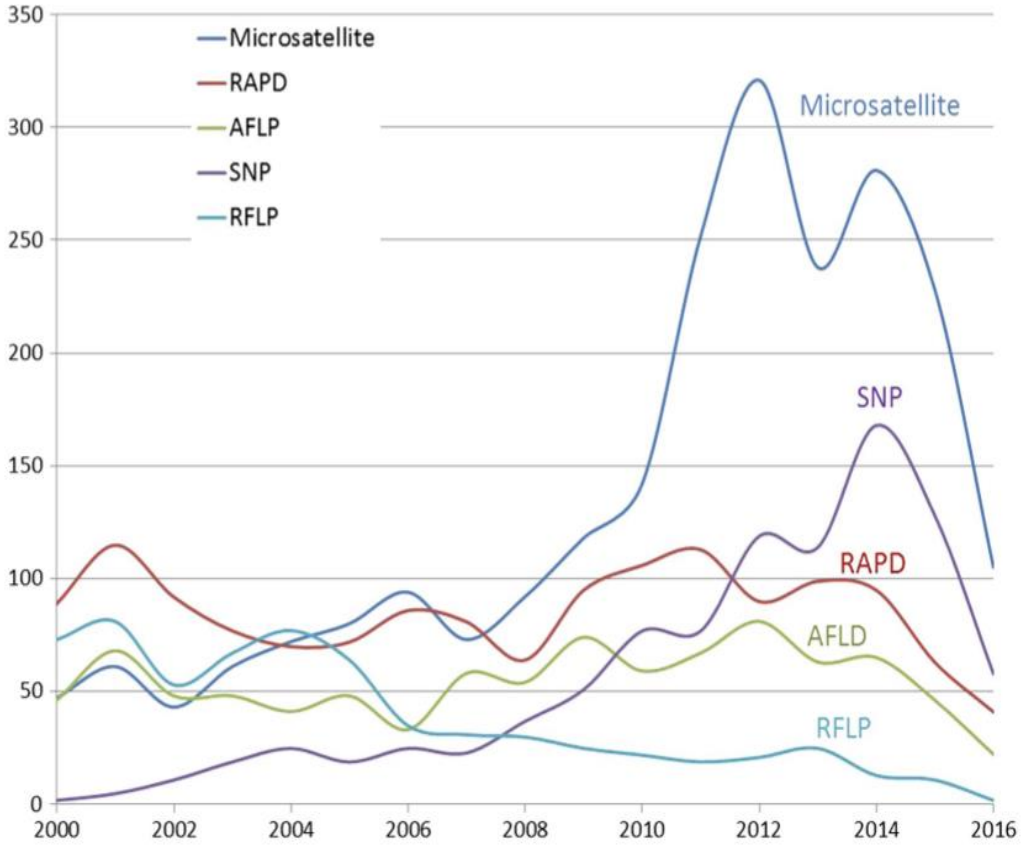

Figure 2: Trends in the use of different molecular markers from the year 2000 to 2016 (Antonio et al., 2017)

Figure 2 shows how microsatellites have gained popularity in recent years as a widely used molecular marker. Keerthi et al. (2018) report that SSR polymorphisms are co-dominant, and their markers are widespread across the genomes of almost all species, making them abundant in almost all of them making them advantageous. Thus, SSR markers will also be used for germplasm characterisation of *Lablab purpureus*.

Identification of the rhizosphere bacteria that are in close proximity to the roots of *Lablab purpureus* varieties is also of great importance.

RHIZOSPHERE

The rhizosphere is the fraction of soil around the plant roots (Figure 3), where microorganisms greatly impact plant health and nutrition (Berg et al., 2006). Microorganisms in the rhizosphere are highly diverse by more than 10-100 times when compared to the bulk soil. These microbes can be neutral to their host plant's health, harmful or beneficial. Plant growth-promoting rhizobacteria (PGPR) are the latter, which are of great importance (Barghouthi, 2011).

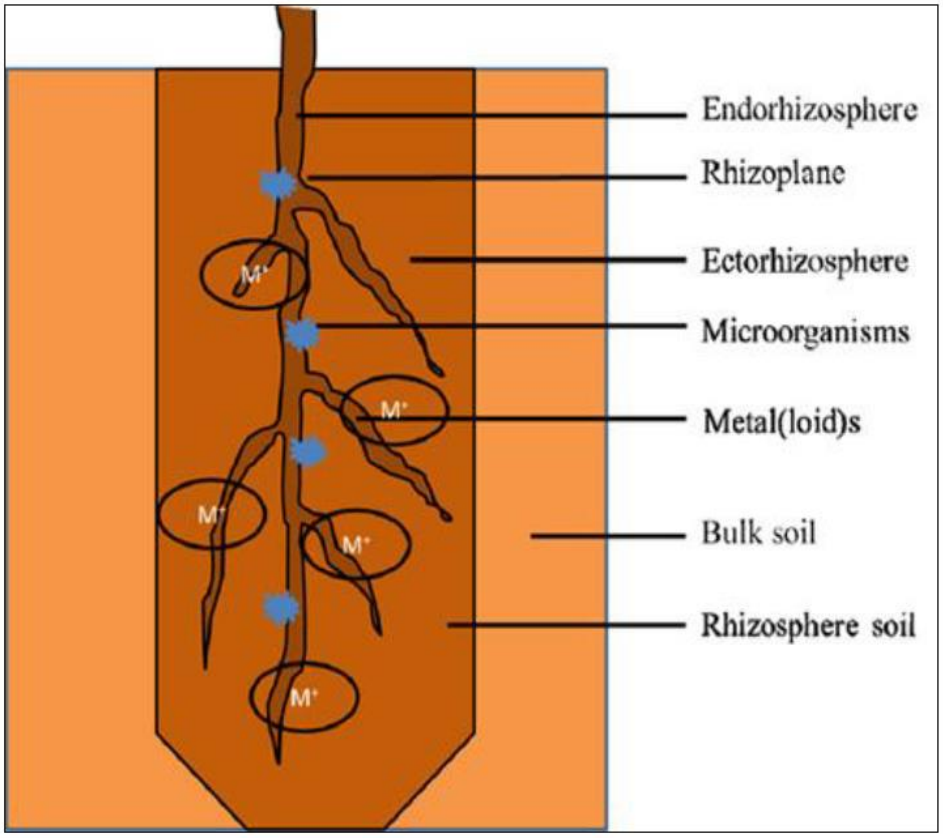

Figure 3: Diagram of rhizosphere (Nazir et al., 2016)

Rhizosphere Bacteria

Rhizobacteria involved in plant growth promotion do so directly by regulating levels of plant hormones or helping in procurement of essential minerals, phosphorus, and nitrogen; or indirectly as biocontrol agents that decrease repressive effects that different pathogens have on plant growth (Ahemad & Kibret, 2014). They improve plant yield and growth when applied to seeds or crops and are usually mediated by root exudates such as siderophores, enzymes, sugars, and amino acids (Chaiharn et al., 2008; Majeed et al., 2015; Wu et al., 2018).

Rhizosphere bacteria are of great importance because they promote plant productivity by regulating nutrient mineralization, acting as environmental buffers, permitting decomposition, and by enhancing water relations (Basu et al., 2021).

With different plant groups and plant communities, the rhizosphere microflora tends to change. Core plant microbiomes depend on soil, soil type, genetics, and environmental factors as well as management interactions (Busby et al., 2017).

Plant Growth Promoting Bacteria

There are beneficial bacteria found in the rhizosphere termed plant growth-promoting bacteria (PGPB). They have been referred to as "biofertilisers" in contemporary agriculture as innovative methods to increase crop productivity and expansion in sustainable agriculture (Omar et al., 2022). PGPB takes possession of the rhizosphere and facilitates plant growth indirectly through biocontrol of the phytopathogens or directly by stimulating the growth as shown in Figure 4 and Figure 5. They do so directly through improved availability of nutrients, including bio-fixation of atmospheric nitrogen, synthesis of phytohormones (such as auxin, cytokinins, gibberellins, and abscisic acid), and solubilization of soil minerals (such as phosphorus and potassium) activity of the enzyme 1-Aminocyclopropane-1-Carboxylate (ACC), production of siderophores (iron sequestration), and in numerous other ways (Omar et

al., 2022). Indirect methods involve competition for nutrients, development of immunity to phytopathogens, which cause soil-borne disease, as well as by the biocontrol of phytopathogens using antibacterial and antifungal biomolecules (Omar et al., 2022). Moreover, PGPB lessens various abiotic and oxidative stresses, and by using them it decreases chemical inputs in environmental pollution and agriculture (Suriyachadkun et al., 2022).

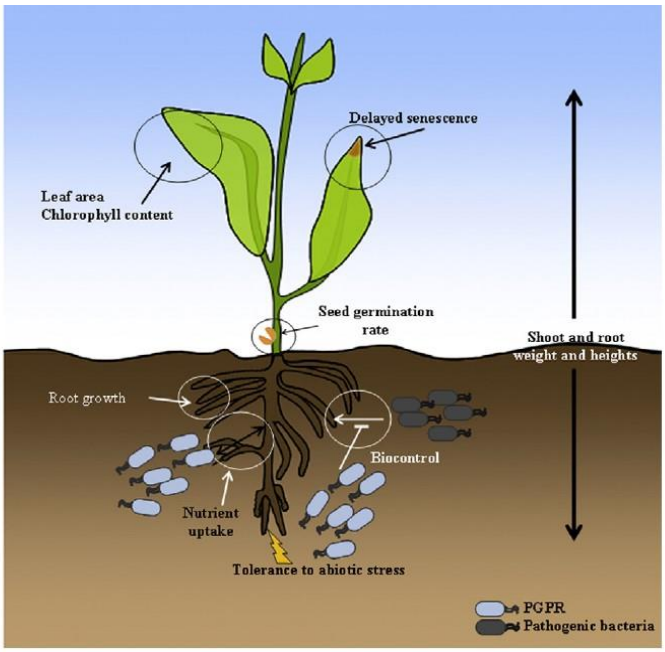

Figure 4: Benefits to plants from host-plant growth promoting rhizobacteria interactions (Pérez-Montaño et al., 2014)

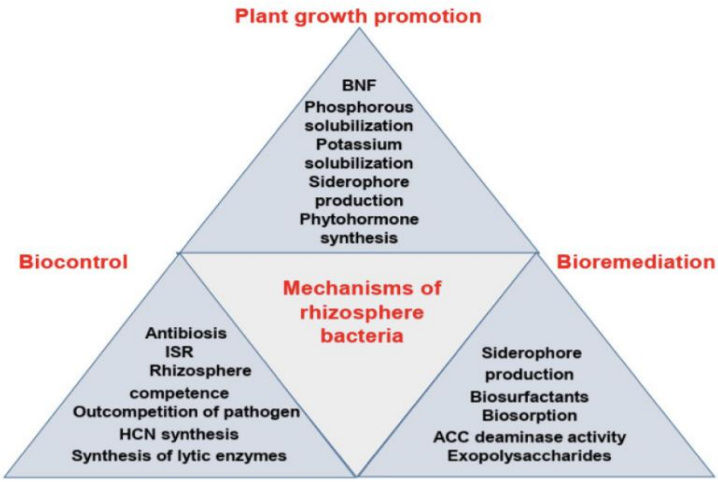

Figure 5: Rhizobacteria's mechanisms for promoting plant growth (Saeed et al., 2021)

There are several methods of identifying PGPB which involve identifying significant bacterial traits, including the production of phytohormones, biological fixation of nitrogen, ACC deaminase activity, production of siderophores and phosphate solubilization (de Souza et al., 2015). Biochemical tests like catalase tests are done to study the presence of the catalase enzyme. Bacterial strains with catalase activity are of importance because they are highly resistant to mechanical, chemical, and environmental stress; such stresses could be low pH, poor nutrient content in the soil and high day temperatures of above 37°C (Kandjimi et al., 2015). The catalase test involves the application of one drop of 3% hydrogen peroxide to a

bacteria colony on a clean glass slide mixing well using a sterile loop and observing. If there is any effervescence, it is an indication of catalase activity (Kandjimi et al., 2015).

Rhizosphere bacteria can be identified by culturing, isolating, and sequencing of the 16sRNA gene.

SEQUENCING 16SRNA GENE

This gene is found in genomes of all archaea and bacteria and some regions of this gene are highly conserved and are thus similar across different species, but other regions differ among species and are known as variable regions (Figure 6). The latter regions are used to distinguish and classify different types of microorganisms and by sequencing 16s RNA one can identify different types of bacteria (Jo et al., 2016).

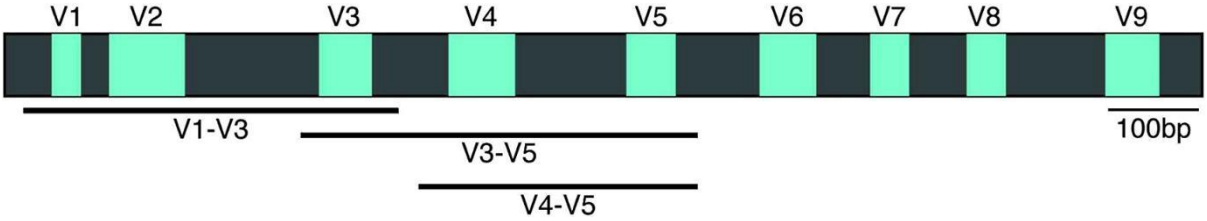

Figure 6: Structure of the 16s RNA gene: Where Neon Green (V1- V9) are variable regions and the dark green are invariable regions (Jo et al., 2016)

When sequencing one must do the following steps: isolate the bacteria, perform bacterial DNA extraction, use PCR to amplify the 16s rRNA gene, sequence a section of the same gene, and then check to see if the sequence matches anything in GenBank and when a match is found, one can generate phylogenetic relatedness (Janda & Abbott, 2007).

Popular sequencing methods are Maxam-Gilbert sequencing, chain-termination method, shotgun sequencing, single molecule real-time sequencing, illumina sequencing (sequence by synthesis), ion torrent sequencing, pyrosequencing, and nanopore sequencing ((Deschamps et al., 2012).

Some limitations and advantages of 16s data are that it is just taxonomical and at a limited resolution and the relative proportions of the bacterial taxa are not quantitative. The advantages are that it is relatively cheap, high throughput with many samples which increases statistical power, and it has well-developed analysis tools and reference databases (Thijs et al., 2017).

Phylogenetic trees can be constructed from the sequence data from 16S rRNA genes. These trees depict evolutionary relationships among microorganisms and give information on various species' genetic diversity and evolutionary history (Tanaseichuk et al., 2014).

PHYLOGENETIC TREES

Phylogenetic trees can be used to organise various types of biological data and to make inferences about possible occurrences in an organism's evolutionary past (Weyenberg & Yoshida, 2015). A phylogenetic tree's bootstrap values show how many times, out of 100, the same branch is seen when the generation of the tree is repeated using a resampled set of data. If we encounter this observation 100 times out of 100, our conclusion is supported. In this scenario, we are certain that the relationship's observed branch is not the result of a single outlying data point (Ojha et al., 2021). With a bootstrap value of 95%, we can reasonably assume that a node is well supported if we recover it in 95 out of 100 iterations of removing one character and resampling our tree. Branches with higher bootstrap values (usually values between 70- 100%) indicate greater confidence that the grouping it represents reflects the true evolutionary relationship among the taxa (Berta et al., 2015). The same authors report that

intermediate values (50-70%) suggest moderate support whereas lower bootstrap values, usually less than 50%, indicate weak support and less confidence in that grouping.

SITUATION IN NAMIBIA

Drought-stricken, semi-arid and arid areas have more severe issues with sustainable agricultural production and thus the use of plant growth promoting bacteria is seen as a valuable bio-resource (Di Benedetto et al., 2019). The bacterial diversity found in the rhizosphere of *Lablab purpureus* varieties in Namibia is not yet known and this legume is beneficial to address the issue of protein shortage from food and animal feed. To understand the diversity of bacteria in a specific plant's rhizosphere, the knowledge, characterisation, and identification of these native bacterial populations is required. These can be later used to achieve the desired crop production when used as growth promoting inoculum thus reducing dependency on chemical fertilisers (Di Benedetto et al., 2019). In this study, bacteria in the rhizosphere of *Lablab purpureus* will be sampled, characterised, screened and thereafter, isolates identified by 16s rRNA gene sequence analysis.

It is of utmost importance to use SSR primers to determine the genetic diversity of *Lablab purpureus* in Namibia. The finding of the study would help in the selection of appropriate germplasm for crop improvement and are useful in creating future breeding programs. The study aims to perform molecular characterisation using microsatellite molecular markers as a starting point for the establishment of breeding programs for the species in Namibia.

REFERENCES

Ahemad, M., & Kibret, M. (2014). Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. *Journal of King Saud University Science*, *26*(1), 1–20. https://doi.org/10.1016/j.jksus.2013.05.001

Antonio, J., Cardenas, G., Mesa, C., Francisco, V., & Agugliaro, M. (2017). Trends in plant research using molecular markers. *Planta*. https://doi.org/10.1007/s00425-017-2829-y

Barghouthi, S. A. (2011). A Universal Method for the Identification of Bacteria Based on General PCR Primers. *Indian Journal of Microbiology*, *51*(4), 430–444. https://doi.org/10.1007/s12088-011-0122-5

Basu, A., Prasad, P., Das, S. N., Kalam, S., Sayyed, R. Z., Reddy, M. S., & Enshasy, H. El. (2021). Plant Growth Promoting Rhizobacteria (PGPR) as Green Bioinoculants: Recent Developments, Constraints, and Prospects. *Sustainability*, *13*(1140), 1–20.

Berg, G., Opelt, K., Zachow, C., Lottmann, J., Götz, M., Costa, R., & Smalla, K. (2006). The rhizosphere effect on bacteria antagonistic towards the pathogenic fungus Verticillium differs depending on plant species and site. *FEMS Microbiology Ecology*, *56*(2), 250–261. https://doi.org/10.1111/j.1574-6941.2005.00025.x

Berta, A., Sumich, J. L., & Kovacs, K. M. (2015). Phylogeny, Taxonomy, and Classification. In *Marine Mammals* (pp. 17–34). Elsevier. https://doi.org/10.1016/b978-0-12-397002-2.00002-8

Busby, P. E., Soman, C., Wagner, M. R., Friesen, M. L., Kremer, J., Bennett, A., Morsy, M., Eisen, J. A., Leach, J. E., & Dangl, J. L. (2017). Research priorities for harnessing plant microbiomes in sustainable agriculture. *PLoS Biology*, *15*(3). https://doi.org/10.1371/journal.pbio.2001793

Chaiharn, M., Chunhaleuchanon, S., Kozo, A., & Lumyong, S. (2008). Screening of Rhizobacteria for Their Plant Growth Promoting Activities. *KMITL Science and Technology Journal*, *8*(1), 18–23.

Chamarthi, S. K., Kumar, A., Vuong, T. D., Blair, M. W., Gaur. P.M, Nguyen, H. T., & Varshney, R. K. (2011). Trait Mapping and Molecular Breeding. In A. Pratap & J. Kumar (Eds.), *Biology and Breeding of Food Legumes* (pp. 296–313).

de Souza, R., Ambrosini, A., & Passaglia, L. M. P. (2015). Plant growth-promoting bacteria as inoculants in agricultural soils. *Genetics and Molecular Biology*, *38*(4), 401–419. https://doi.org/10.1590/S1415-475738420150053

Deschamps, S., Llaca, V., & May, G. D. (2012). Genotyping-by-sequencing in plants. *Biology*, *1*(3), 460–483. https://doi.org/10.3390/biology1030460

Dholakia, H. P., Joshi, M. K., & Delvadiya, I. R. (2019). Molecular characterization of Indian bean (Lablab purpureus L.) genotypes. *Journal of Pharmacognosy and Phytochemistry*, *8*(2), 455–463.

Di Benedetto, N. A., Campaniello, D., Bevilacqua, A., Cataldi, M. P., Sinigaglia, M., Flagella, Z., & Corbo, M. R. (2019). Isolation, screening, and characterization of plant-growth-promoting bacteria from durum wheat rhizosphere to improve N and P nutrient use efficiency. *Microorganisms*, *7*(11), 1–18. https://doi.org/10.3390/microorganisms7110541

Gibson, G., & Spencer, V. M. (2009). *A Primer of Genome Science* (3rd ed.). Sinauer Associates.

Gyang, P. J., Nyaboga, E. N., & Muge, E. K. (2017). Molecular Characterization of Common Bean (Phaseolus vulgaris L.) Genotypes Using Microsatellite Markers. *Journal of Advances in Biology & Biotechnology*, *13*(2), 1–15. https://doi.org/10.9734/JABB/2017/33519

Janda, J. M., & Abbott, S. L. (2007). 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: Pluses, perils, and pitfalls. *Journal of Clinical Microbiology*, *45*(9), 2761–2764. https://doi.org/10.1128/JCM.01228-07

Jaskulak, M., Rorat, A., Vandenbulcke, F., Pauwels, M., Grzmil, P., & Plytycz, B. (2022). Polymorphic microsatellite markers demonstrate hybridization and interspecific gene flow between lumbricid earthworm species, Eisenia andrei and E. fetida. *Plos one*, *17*(2), e0262493. https://doi.org/10.1371/journal.pone.0262493

Jo, J. H., Kennedy, E. A., & Kong, H. H. (2016). Research Techniques Made Simple: Bacterial 16S Ribosomal RNA Gene Sequencing in Cutaneous Research. *Journal of Investigative Dermatology*, *136*, e23–e27. https://doi.org/10.1016/j.jid.2016.01.005

Kamotho, G. N., Kinyua, M. G., Muasya, R. M., Gichuki, S. T., & Wanjala, B. W. (2016). Assessment of Genetic Diversity of Kenyan Dolichos Bean (Lablab purpureus L. Sweet) Using Simple Sequence Repeat (SSR) Markers. *International Journal of Agriculture, Environment and Bioresearch*, *1*(01), 26–43.

Kandjimi, O. S., Uzabakiriho, J.-D., & Chimwamurombe, P. M. (2015). Isolation and characterization of culturable bacteria from bulk soil samples and the rhizosphere of arid-adapted Tylosema esculentum (Burchell). A. Schreiber (Marama bean) in Namibia. *African Journal of Biotechnology*, *14*(11), 944–952. https://doi.org/10.5897/AJB2014.14257

Keerthi, C. M., Ramesh, S., Byregowda, M., & Vaijayanthi, P. V. (2018). Simple Sequence Repeat (SSR) Marker Assay-Based Genetic Diversity among Dolichos Bean (Lablab purpureus L. Sweet) Advanced Breeding Lines Differing for Productivity per se Traits. *International Journal of Current Microbiology and Applied Sciences*, *7*(05), 3736–3744. https://doi.org/10.20546/ijcmas.2018.705.433

Majeed, A., Kaleem Abbasi, M., Hameed, S., Imran, A., & Rahim, N. (2015). Isolation and characterization of plant growth-promoting rhizobacteria from wheat rhizosphere and their effect on plant growth promotion. *Frontiers in Microbiology*, *6*(MAR). https://doi.org/10.3389/FMICB.2015.00198

McBenedict, B., Chimwamurombe, P., Kwembeya, E., & Maggs-kölling, G. (2016). Genetic Diversity of Namibian Pennisetum glaucum (L.) R. BR. (Pearl Millet) Landraces

Analyzed by SSR and Morphological Markers. *The Scientific World Journal*, *2016*, 1–11.

Minde, J. J., Venkataramana, P. B., & Matemu, A. O. (2020). Dolichos Lablab-an underutilized crop with future potentials for food and nutrition security: a review. *Critical Reviews in Food Science and Nutrition*. https://doi.org/10.1080/10408398.2020.1775173

Moteetee, A. N., & Van Wyk, B. E. (2012). A revision of the genus Dolichos (Fabaceae, Papilionoideae, Phaseoleae), including Lesotho and Swaziland. *South African Journal of Botany*, *78*, 178–194. https://doi.org/10.1016/j.sajb.2011.06.012

Murphy, A. M., & Colucci, P. E. (1999). A tropical forage solution to poor quality ruminant diets: A review of Lablab purpureus. *Livestock Research for Rural Development*, *11*(2), 96–113.

Nazir, N., Kamili, A., Zargar, M., Khan, I., Shah, D., Parray, J., & Tyub, S. (2016). Effect of Root Exudates on Rhizosphere Soil Microbial Communities. *ResearchGate*, *16*, 88–95. https://www.researchgate.net/publication/331876243

Ojha, K. K., Mishra, S., & Singh, V. K. (2021). Computational molecular phylogeny: concepts and applications. In *Bioinformatics: Methods and Applications* (pp. 67–89). Elsevier. https://doi.org/10.1016/B978-0-323-89775-4.00025-0

Omar, A. F., Abdelmageed, A. H. A., Al-Turki, A., Abdelhameid, N. M., Sayyed, R. Z., & Rehan, M. (2022). Exploring the Plant Growth-Promotion of Four Streptomyces Strains from Rhizosphere Soil to Enhance Cucumber Growth and Yield. *Plants*, *11*(23). https://doi.org/10.3390/plants11233316

Pérez-Montaño, F., Alías-Villegas, C., Bellogín, R. A., Del Cerro, P., Espuny, M. R., Jiménez-Guerrero, I., López-Baena, F. J., Ollero, F. J., & Cubo, T. (2014). Plant growth promotion in cereal and leguminous agricultural important plants: From microorganism capacities to crop production. *Microbiological Research*, *169*(5–6), 325–336. https://doi.org/10.1016/j.micres.2013.09.011

Raghu, B. R., Samuel, D. K., N, M., & S, A. T. (2018). Dolichos Bean : An underutilized and unexplored crop with immense potential. *International Journal of Recent Advances in Multidisciplinary Research*, *5*(12), 4338–4341.

Rai, N., Kumar, A., Singh, P. K., Singh, M., Datta, D., & Rai, M. (2010). Genetic relationship among Hyacinth bean (Lablab purpureus) genotypes cultivars from different races based on quantitative traits and random amplified polymorphic DNA marker. *African Journal of Biotechnology*, *9*(2), 137–144. https://doi.org/10.4314/ajb.v9i2.

Rezaei, N., & Hedayat, M. (2013). Allele Frequency. *Elsevier*, *1*, 77–78. https://doi.org/10.1016/B978-0-12-374984-0.00032-2

Saeed, Q., Xiukang, W., Haider, F. U., Kučerik, J., Mumtaz, M. Z., Holatko, J., Naseem, M., Kintl, A., Ejaz, M., Naveed, M., Brtnicky, M., & Mustafa, A. (2021). Rhizosphere bacteria in plant growth promotion, biocontrol, and bioremediation of contaminated sites: A comprehensive review of effects and mechanisms. *International Journal of Molecular Sciences*, *22*(19). https://doi.org/10.3390/ijms221910529

Sheahan, C. M. (2012). *Lablab (Lablab purpureus) Plant Guide*. http://plants.usda.gov/plantguide/pdf/pg_lapu6.pdf

Sserumaga, J. P., Kayondo, S. I., Kigozi, A., Kiggundu, M., Namazzi, C., Walusimbi, K., Bugeza, J., Molly, A., & Mugerwa, S. (2021). Genome-wide diversity and structure variation among lablab [Lablab purpureus (L.) Sweet] accessions and their implication in a Forage breeding program. In *Genetic Resources and Crop Evolution*. https://doi.org/10.1007/s10722-021-01171-y

Suriyachadkun, C., Chunhachart, O., Srithaworn, M., Tangchitcharoenkhul, R., & Tangjitjareonkun, J. (2022). Zinc-Solubilizing Streptomyces spp. as Bioinoculants for

Promoting the Growth of Soybean (Glycine max (L.) Merrill). *Journal of Microbiology and Biotechnology*, *32*(11), 1435–1446. https://doi.org/10.4014/jmb.2206.06058

Tanaseichuk, O., Borneman, J., & Jiang, T. (2014). Phylogeny-based classification of microbial communities. *Bioinformatics*, *30*(4), 449–456. https://doi.org/10.1093/bioinformatics/btt700

Thijs, S., De Beeck, M. O., Beckers, B., Truyens, S., Stevens, V., Van Hamme, J. D., Weyens, N., & Vangronsveld, J. (2017). Comparative evaluation of four bacteria-specific primer pairs for 16S rRNA gene surveys. *Frontiers in Microbiology*, *8*, 494. https://doi.org/10.3389/fmicb.2017.00494

Wang, M. L., Morris, J. B., Barkley, N. A., Dean, R. E., Jenkins, T. M., Pederson, G. A., Morris, J. B., Barkley, N. A., Dean, R. E., Jenkins, T. M., & Pederson, G. A. (2016). Evaluation of genetic diversity of the USDA Lablab purpureus germplasm collection using simple sequence repeat markers. *Journal of Horticultural Science and Biotechnology*, *82*(4), 571–578. https://doi.org/10.1080/14620316.2007.11512275

Weyenberg, G., & Yoshida, R. (2015). Reconstructing the Phylogeny: Computational Methods. In *Algebraic and Discrete Mathematical Methods for Modern Biology* (pp. 293–319). Elsevier. https://doi.org/10.1016/B978-0-12-801213-0.00012-5

Wu, Z., Liu, Q., Li, Z., Cheng, W., Sun, J., Guo, Z., Li, Y., Zhou, J., Meng, D., Li, H., Lei, P., & Yin, H. (2018). Environmental factors shaping the diversity of bacterial communities that promote rice production. *BMC Microbiology*, *18*(1), 1–11. https://doi.org/10.1186/s12866-018-1174-z

Zhang, Z., & Lijuan, Z. (2013). Molecular characterization of genetic diversity of underutilized crops: Buckwheat as an example. *Acta Horticulturae*, *979*, 407–420. https://doi.org/10.17660/actahortic.2013.979.44