



Full Length Article

Investigating the Impact of Soil pH and Texture on Legume Species Root Nodule Formation

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Abstract

Nodule formation plays a pivotal role in legume plants establishing a mutualistic symbiotic relationship that can reduce the need for nitrogen fertilizer application. The objective of this study was to determine the effect of texture, soil pH and plant growth phase on the formation of root nodules of various legume species. This study, conducted in Padang City, West Sumatra, Indonesia, from January to February 2024, employed qualitative tests and observational methods to investigate the impact of soil texture, pH, and plant growth phase on root nodule formation across various legume species. Eight types of legumes served as research subjects including five wild varieties and three domestic types. At each location eight plants were sampled, enclosed in tightly sealed plastic bags with open tops and transported to the laboratory ensuring that soil moisture was maintained at field capacity. Spearman's Rho correlation analysis established relationships between variables at the 5% significance level. Regression models were examined based on the correlation coefficient of determination (R^2). The regression equation displaying the highest correlation coefficient was selected. The formation of root nodules on legume plants' roots is influenced by various soil factors. The shape of the nodule is influenced by the legume leaves' shape. Sandy loam texture produces more nodules than other soil textures. The highest number of nodules is found in the primordial phase of the flower. A soil pH close to 5 produces more nodules than lower or higher soil pH levels. Based on the multiple linear regression equation, it is known that the formation of legume root nodules will increase as root weight and soil pH increase. The formation of effective root nodules will decrease as soil pH increases beyond a certain level. In general, the number of root nodules and effective root nodules are directly proportional to soil texture. Sandy clay soil is conducive to higher nodule formation, followed by clay texture and silt loam clay. The effectiveness of nodules was nearly consistent across each soil texture but reached its peak in clay-textured soil. Sandy-textured soil produces legume plants with the highest nodule formation compared to clay soil and exhibits high levels of organic matter. Generally, sandy soil has the potential for up to twice the nodule formation compared to clay soil and six times more than organic soil. It has been demonstrated that sandy soil possesses a higher macro-pore content than clay soil. However, organic soil also has high macro-pore content, along with optimal macro pores. Despite the presence of abundant organic matter, organic soil is not capable of producing more nodules than sandy or clay-textured soil.

Keywords: Growth phase; Legume; Root nodules; Soil pH; Soil texture; West Sumatra

Introduction

Soil fertility is determined by sufficient levels of organic matter, minerals, water and air in accordance with their respective proportions. This proportion is also greatly

influenced by soil texture (Mariati *et al.* 2022). There is a growing interest in comprehending how cultivation practices, plant genotype, climate and soil conditions collectively influence the establishment of root nodule bacterial communities in legumes (Ramoneda *et al.* 2020).

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Legume crops are a good source of food, feed and fodder, and they are grown on large scale in the arid, semi-arid tropics and tropical climates (Ahmed *et al.* 2022). As stated by Li *et al.* (2022), legumes, unlike most land plants, can form root nodules that establish symbiotic relationships with nitrogen-fixing bacteria, enabling them to secure nitrogen for growth. Fertile soil is usually characterized by a neutral soil chemical reaction (pH), optimal availability of nutrients for plants and various types of microorganisms that benefit plant growth. Wendlandt *et al.* (2022) explains that legumes are plants capable of enhancing soil fertility through the establishment of a symbiotic relationship with nitrogen-fixing *Rhizobium* bacteria in the atmosphere. Nitrogen is abundant in the atmosphere (~79% as N₂ gas). However, most plants are unable to directly utilize atmospheric nitrogen. Becker *et al.* (2023) demonstrated the inoculation effects on nodulation and biological N₂ fixation (BNF) of *Vigna unguiculata* (L.) Walp. (Fabaceae), along with consequent impacts on carbon (C) and nitrogen (N) pools. The development or cultivation of legume plants is also recommended for crop rotation patterns on land to increase soil fertility based on their nodule formation ability. Various species of legumes have been used by humans as a source of vegetable protein. Legume leaves that grow wild have even been used as forage for livestock, which has high protein content.

The formation of legume-*Rhizobium* symbiosis results from mutual recognition between microorganisms and plants, as well as interactions among signaling molecules. First, flavonoid compounds released by legume roots induce rhizobia to produce Nod factors can be recognized by receptors in legume plants. Then, the root hairs of leguminous plants curl, change shape and form infection threads (Chaulagain and Frugoli 2021) through which rhizobia penetrate the root tissue. Simultaneously, several root cortical cells are stimulated to begin dividing to form primordial nodules. Cells infected with bacteria originate from cortex cells in the root undergo changes, and then multiply to form the nodule meristem. The timing of initiation development and maturation of nodule organogenesis within root cells has been well documented (Zhou *et al.* 2021).

Some plant species establish mutualistic symbiotic relationships with nitrogen-fixing bacteria to address N deficiency. The interaction between leguminous plants and rhizobial bacteria is a canonical example of such mutualism. This leads to the formation of root nodules, which provide an environment for bacteria to convert atmospheric dinitrogen (N₂) into ammonia (NH₄⁺). Nodule formation is initiated by a compound secreted by *Rhizobium* called nodulation factor (Nod) (Millar *et al.* 2023). Perception of Nod factors by receptors in plant root cells initiates nodule organogenesis and is important for bacterial infection. Some legumes have such a narrow host range that only one or a few *Rhizobium* species can initiate nodulation. The Nod factor receptor largely determines this specificity. Structural

characterization of the Nod factor receptor binding site in legumes provided insight into the structural basis of rhizobial Nod factor recognition, key to understanding the evolution of specificity in symbiosis (Chaulagain and Frugoli 2021).

According to Wendlandt *et al.* (2022), soil N levels positively predict the number of nodules formed on host plants. However, this association may be attributed to variation in plant genotypes affecting nodule formation rather than differences between soil microbial communities. The loose soil texture caused by the high content of organic matter (OM) and sand will help the roots develop and penetrate deeper. Good roots will also make it easier for *Rhizobium* bacteria to form root nodules on their host plants (Ejeagba *et al.* 2023). It is still necessary to understand the importance of identifying soil texture in relation to nodule formation on the roots of legume plants. We hypothesize that an increase in soil pH and looser soil conditions will lead to a greater formation of legume root nodules. However, it is still not certain about the relationship between pH, texture and the growth phase of legume plants and the formation of root nodules. Nevertheless, legumes are also able to adapt to marginal soil conditions, resulting in the formation of root nodules. It is necessary to carry out exploration to find out the various characteristics of legume plants, both wild and domestic (maintained). The aim of the study was to determine the effect of texture, soil pH and plant growth phase on the formation of root nodules of various legume species.

Materials and Methods

Research area and material

The research was conducted from January to February 2024 in Padang City, West Sumatra Province, Indonesia. The study site was Padang City in a tropical climate, located at latitude -0.947 and longitude 100.417, encompassing several sub-districts, namely: Koto Tengah (A) at latitude -0.225 and longitude 100.417, Kuranji (B) at latitude -0.902 and longitude 100.439, Nanggalo (C) at latitude -1.220 and longitude 100.458 and Padang Utara (D) at latitude -0.897 and longitude 100.349 (Fig. 1).

Procedures

This research employed a qualitative research design with an exploratory descriptive method. The objective was to explore various types of legumes growing in four sub-districts in Padang City. Sampling of legume plants was conducted randomly across several sub-districts. Eight types of legumes served as research objects, including five types of wild legumes (*Mimosa invisa* Mart. ex Colla, *Crotalaria incana* L., *Calopogonium mucunoides* Desy and Wit. *Macroptillium atropurpureum* (DC.) Urb. and three types of domestic legumes (*Leucaena leucocephala*, *Glycine max* (L.) Merr. *Vigna unguiculata* (L.) Walp. and

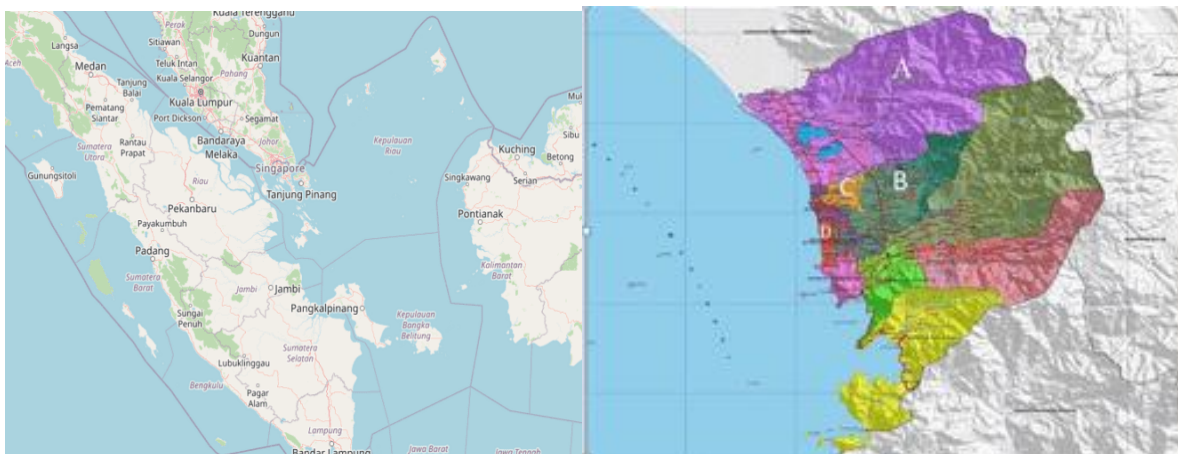


Fig. 1: Padang City, West Sumatra (<https://peta.web.id/map/city/padang-city>)

Arachis hypogaea L.). To collect the legume plants, a shovel was used to dig up the plants completely, including the roots and rhizosphere soil, which were then transported to the laboratory. Five plants were sampled at each location (Fig. 1) and placed in tightly closed plastic bags with open tops. The soil and plant samples' moisture were maintained at field capacity during transportation to the laboratory.

The tools utilized in this research included earth-digging tools, shovels, a cell phone camera (Samsung M2), analytical scales (AND HF-300), umbrella paper, rolled tissue, cotton, gauze, label paper, pH meter, and plastic wrap. The materials comprised active and inactive root nodules from legume plants, rhizosphere soil from legume plants, distilled water, and a shaker.

Technique: Plants, previously collected from the field, were carefully prepared by opening the rhizosphere soil attached to their roots, followed by the separation of the soil from the root nodules. Both pH and soil texture of the rhizosphere soil for each legume plant were determined. Soil pH was determined using a pH electrode, with the soil: water ratio maintained at 1:2.5. The mixture was shaken in a shaker bottle for 15 min, and then the pH was measured, following the method outlined by Suda *et al.* (2021). Soil textures were determined through pipette methods, according to (Orzechowski *et al.* 2014). The root nodules are counted on the roots of each plant, the determination of effective root nodules involves calculating the root nodules that are damaged (non-effective) or do not produce red fluid (leg haemoglobin). This is achieved by employing the following formula 1:

$$\text{Effective root nodules} = \frac{(\text{total nodules} - \text{non-effective nodules})}{\text{Total nodules}} \times 100\% \quad (1)$$

The total nodules, non-effective nodules and effective root nodules are key parameters for the assessment of nodulation in legume plants.

Statistical analysis

Exploratory data analysis was carried out at a predetermined

location followed by the analysis of pH the number of root nodules and the percentage of effective nodules. The analysis in this research combines qualitative descriptive methods and quantitative F-tests and uses further analysis with least significant Difference (LSD) at a significance level of $P < 0.05$. This study employed Spearman's Rho Correlation Analysis to establish relationships between variables at a $P < 0.05$. The observed variables included the number of roots, root weight, and the number of nodules, Effective nodule (%), soil pH and texture. The analysis results were presented graphically. Multiple linear regression models were utilized to determine equation 2. Describing the relationship between soil pH texture and the number of root nodules, as well as the effective root nodules identified by the highest correlation coefficient (r) or the coefficient of determination (r^2). Multiple linier regression, captures the relationship between multiple independent variables and dependent variable (y) (number of root nodule or effective root nodule).

$$\text{Equation; } y = \beta_0 + \beta_1(\text{soil pH}) + \beta_2(\text{soil texture}) + \beta_3(\text{root weight}) + \dots + \epsilon \quad (2)$$

Where: β_0 is the intercept; β_1 , β_2 and β_3 are the coefficients for the respective independent variables (e.g., soil pH soil texture root weight); ϵ represents the error term.

Results

Nodule characteristics are based on the legume plant species. The species of legume plant greatly determines the shape of the nodules produced. In general, the species *C. mucunoides* and *V. unguiculata* have nodules with a more rounded shape, while *M. invisa* produces nodules that are flatter, elongated and smaller (Fig. 2, 3). Based on the legume species, it was found that *V. unguiculata* produced the highest number of root nodules, followed by *M. pudica* and *C. incana*, while the others had fewer root nodules. Similarly, *M. atropurpureum* produced the highest percentage of effective nodules and were not significantly different from the legume *V. unguiculata* (Table 1).

Table 1: The relationship between legume species and their ability to form nodules, and effective nodule

Species	Number of root nodule	Effective nodule (%)
<i>Mimosa pudica</i>	143.42 ± 9.93b	88.12 ± 10.02a-c
<i>Crotalaria incana</i>	125.50 ± 13.82c	80.15 ± 6.05bc
<i>Leucaena leucocephala</i>	32.47 ± 4.14e	75.10 ± 12.6c
<i>Vigna unguiculata</i>	228.53 ± 12.14a	96.56 ± 1.45a
<i>Arachis hypogaea</i>	56.33 ± 11.98d	94.51 ± 4.80ab
<i>Calopogonium mucunoides</i>	18.66 ± 4.83f	83.33 ± 1.65bc
<i>Glicine max</i>	51.00 ± 6.09d	83.95 ± 2.95a-c
<i>Macroptilium atropurpureum</i>	33.33 ± 3.36e	96.98 ± 1.70a

Means ± standard deviations sharing same letter differ non-significantly ($P > 0.05$)

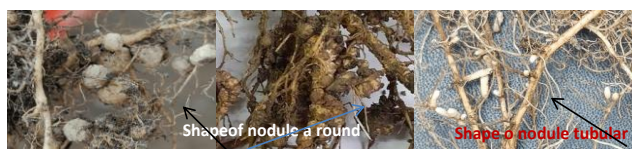


Fig. 2: Appearance of the nodule shape of the legume plants *C. mucunoides*, *V. unguiculata* and *M. invisa*

No	Species	Roots with nodule of legumes	Shoot of legumes
1.	<i>Mimosa invisa</i>		
2.	<i>Crotalaria incana</i>		
3.	<i>Leucaena leucocephala</i>		
4.	<i>Glicine max</i>		
5.	<i>Vigna unguiculata</i>		
6.	<i>Arachis hypogaea</i>		
7.	<i>Calopogonium mucunoides</i>		
8.	<i>Macroptilium atropurpureum</i>		

Fig. 3: Various Nodule and Plant Shapes of Various Legume Species

Table 2: The relationship between growth phase of legume and their ability to form nodules, and effective nodules

Growth phase	Number of root nodule	Effective nodule (%)
Vegetative	129.20 ± 16.39b	89.52 ± 13.69a
Flower primordial	193.40 ± 42.43a	76.15 ± 11.55a
Pod formation	59.20 ± 36.59c	86.05 ± 06a

Means ± standard deviations sharing same letter differ non-significantly ($P > 0.05$)

Table 3: The relationship between soil texture and the number of roots, the number of nodules, and the effectiveness of nodules in legumes

Soil texture	Number of root nodules	Number of root nodule	Effective nodule (%)
Sandy loam	14.25 ± 3.86a	184.00 ± 19.14a	90.77 ± 10.54a
Loam	13.00 ± 1.22a	114.20 ± 3.27b	86.57 ± 12.72a
silt loam	9.5 ± 5.92b	61.20 ± 8.32c	84.76 ± 8.37a

Means ± standard deviations sharing same letter differ non-significantly ($P > 0.05$)

Table 4: Presents the correlation between various soil factors and the formation of root nodules in legume

			Texture	Nodule total	Effective (%)	pH	root	
							total	weight
Spearman's Rho	Texture	Correlation Coefficient	1.000	0.285	0.156	0.692	0.241	0.345
		Sig. (2-tailed)	0.00	0.251	0.537	0.301	0.336	0.161
	Sum of nodule	Correlation Coefficient	0.285	1.000	0.259	-0.770**	0.377	0.655**
		Sig. (2-tailed)	0.251	0.00	0.299	0.009	0.123	0.003
	% effective	Correlation Coefficient	0.156	0.259	1.000	-0.002	0.187	0.141
		Sig. (2-tailed)	0.537	0.299	0.00	0.994	0.456	0.576
	pH	Correlation Coefficient	0.692	-0.770**	-0.002	1.000	-0.234	-0.116
		Sig. (2-tailed)	0.301	0.009	0.994	0.00	0.349	0.648
	Sum of root number	Correlation Coefficient	0.241	0.377	0.187	-0.234	1.000	0.445
		Sig. (2-tailed)	0.336	0.123	0.456	0.349	0.00	0.065
	Weight of root (g)	Correlation Coefficient	0.345	0.655**	0.141	-0.116	0.445	1.000
		Sig. (2-tailed)	0.161	0.003	0.576	0.648	0.065	0.00

**Correlation is significant at the 0.01 level (2-tailed)

Table 5: Relationship between pH and soil texture on the number of root nodules in legume

Variable	Coefficient Regression	T Statistic	P Value	R Square	F
(Constant)	381.986	1.559	0.140	0.223	0.151 ^b
pH	-84.519	-3.521	0.009		
Texture	84.092	3.048	0.038		

Plant growth phase and formation of legume root nodules

Based on the plant growth phase the highest nodule formation was observed in the flower primordial phase. Nodule formation during the vegetative phase and the pod formation phase was nearly equal in terms of the number of root nodules produced. Effective root nodule formation was not influenced by the plant growth phase, as depicted in Table 2.

The outcomes of the correlation statistical analysis are outlined in Table 4. A significant relationship was observed between pH, root weight, and the number of root nodules in legume plants at the 5% level of significance. However other variables showed non-significant relationships.

Following additional tests to assess the impact of soil texture and pH on the number of root nodules in each species, multiple linear analysis was conducted, and the results are presented in Table 5. The multiple linear relationships are expressed in Eq. 3.

$$\text{Number of Root Nodules} = 381.986 - 84.519 \text{ pH} + 84.092 \text{ texture (3)}$$

The regression model presented in Equation 2 can be interpreted as follows: The regression coefficient with a negative sign of 84.519 indicates a negative relationship between soil pH and the number of nodules. This implies that as the soil pH increases, the number of nodules decreases. For every 1% increase in soil pH, the number of nodules is expected to decrease by 84 units, assuming the soil texture for each species remains constant. On the other hand, the positive regression coefficient of 84.092 signifies a positive relationship between soil texture and the number of nodules. Specifically, the number of nodules in sandy loam soil texture is greater than in silt loam and clay loam, and the number of nodules in silt loam soil texture is greater than in clay. Consequently, the highest number of nodules is anticipated in sandy clay soil texture compared to other soil textures, assuming the soil pH in each species remains constant.

The respective significance values are 0.009 and 0.038, both below the 5% significance level, indicating a partially significant influence of both pH and soil texture on the number of nodules. However, the significance value in the F test is 0.151, which is greater than the 5% significance

Table 6: Relationship between pH and soil texture on effective root nodules (%)

Variable	Coefficient Regression	T Statistic	P Value	R Square	F
(Constant)	98.477	3.833	0.002	0.205	0.675
pH	-3.645	-0.626	0.541		
Texture	3.850	0.895	0.385		

Table 7: Relationship between legume root weight and soil pH on the number of root nodules

Variable	Coefficient Regression	T Statistic	P Value	R Square	F
(Constant)	-41.534	-0.207	0.839	0.445	0.001
Weight of root	11.774	3.372	0.004		
pH	13.865	3.365	0.020		

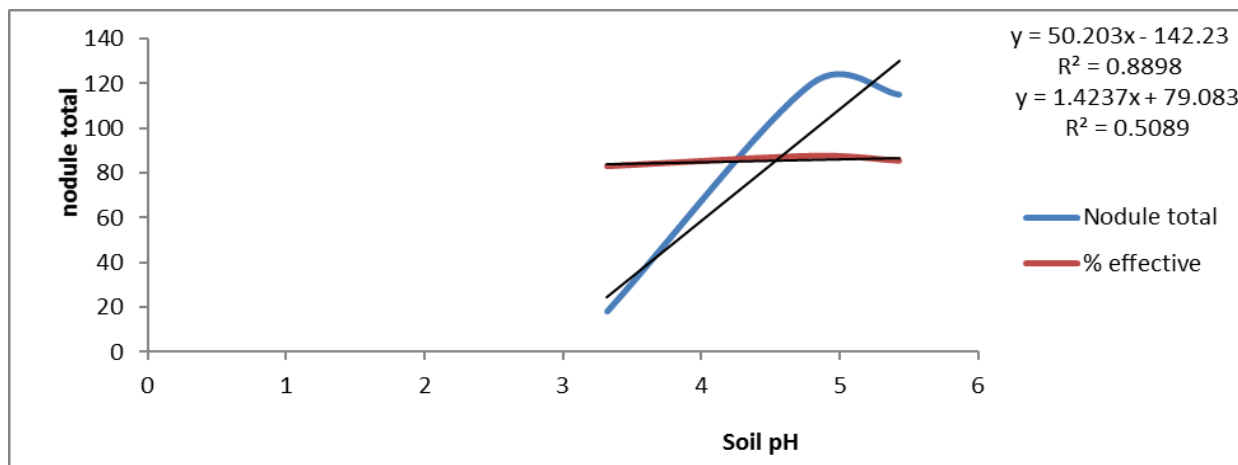


Fig. 4: Bar diagram of relationship between soil pH and nodule formation in legumes

level. Therefore, pH and texture simultaneously do not have a significant effect on the number of nodules. The coefficient of determination value is 0.223, indicating that pH and texture collectively explain 22.3% of the factors influencing the number of nodules, with the remaining factors not considered in this research.

Moving on to further tests assessing the effect of soil texture and pH in each species on effective root nodules (%) using multiple linear analysis, the results are presented in Table 5, along with the multiple linear relationships in Eq. 4.

$$\text{Effective Root Nodule (\%)} = 98.477 - 3.645 \text{ pH} + 3.850 \text{ texture} \quad (4).$$

The regression model described in Equation 3 can be interpreted as follows: The regression coefficient with a negative value of -3.645 indicates a negative relationship between soil pH and the percentage of effective nodules. In other words, as the soil pH increases, the percentage of effective nodules decreases. For every 1 unit increase in soil pH the percentage of effective nodules is expected to decrease by 3.645 units assuming the soil texture for each species remains constant. On the other hand, the positive regression coefficient of 3.850 suggests a positive relationship between soil texture and the percentage of effective nodules. Specifically, the percentage of effective nodules is highest in loamy sand, followed by silt loam and

loam soil textures. Additionally, within silt loam, the percentage of effective nodules is greater than in loam soil texture, assuming that the soil pH in each species remains constant.

The respective significance values for pH and soil texture are 0.542 and 0.385, both exceeding the 5% significance level. Consequently, there is no partial significance observed between pH and soil texture regarding the percentage of effective nodules. Furthermore, the significance value in the F test is 0.675, which is greater than the 5% significance level. Thus, pH and texture simultaneously do not have a significant effect on the percentage of effective nodules. The coefficient of determination value is 0.205, indicating that pH and texture collectively explain 20.5% of the factors influencing the percentage of effective nodules, with the remaining factors not considered in this research.

Following additional tests to assess the impact of legume root weight and soil pH on the number of root nodules in each species, multiple linear analysis was conducted, and the results are presented in Table 7. The multiple linear relationships are expressed in Equation 5.

$$\text{Number of Root Nodule} = -41.534 + 11.774 \text{ root weight} + 13.865 \text{ pH} \quad (5).$$

The regression model presented in Table 7 can be explained as follows: The regression coefficient value of 11.774 indicates a positive relationship between the weight

of legume roots and the number of nodules. As the root weight of the legume increases, the number of nodules also increases, assuming that the soil texture and pH for each species remain constant. Additionally, the regression coefficient value of 13.865 suggests a positive relationship between soil pH and the number of nodules. As the pH increases, the number of nodules tends to increase, assuming that the soil texture for each species is constant.

The respective significant values for pH and root weight were 0.004 and 0.020, both below 5% significance level, indicating a significant influence of pH and weight on the number of nodules. Furthermore, the significance value in the F-test was less than 0.05, suggesting that both pH and weight simultaneously have a significant effect on the number of nodules. The coefficient of determination was 0.445, indicating that pH and legume root weight collectively explain 44.5% of the factors influencing the number of nodules, with the remaining factors not considered in this study.

The relationship between soil pH and the number of root nodules and the percentage of effective root nodules

The formation of root nodules on legume plants' roots is influenced by various soil factors. Based on the exploration results in various sub-districts in the city of Padang. It is evident that there exists a quadratic relationship between soil pH and the formation of nodules, as well as the percentage of effective root nodules. This quadratic relationship is illustrated in Fig. 4, showing a correlation coefficient ($R^2 = 0.889$). The depicted relationship indicates that high soil pH corresponds to plants with a high number of root nodules. However, it is noteworthy that soil pH does not exhibit a relationship with the percentage of effective nodules, as evidenced by a determination coefficient.

Soil texture and root-nodule formation

Soil texture plays a significant role in influencing the number and weight of legume roots. The exploration revealed that the highest number of legume plant roots was observed in sandy loam soil, followed by clay texture and finally, silt loam (Table 3). Similarly, the fresh weight of legume roots was highest in sandy loam texture, followed by silt loam, and finally, in clay soil. Soil texture plays a crucial role in influencing root nodule formation. A coarser texture, such as sandy soil, results in a higher number of root nodules compared to other soil textures. Sandy loam soil has larger particles and is relatively loose, allowing for good drainage and aeration. The effective formation of root nodules is also influenced by soil texture, with clay soil producing the lowest number of effective root nodules compared to sandy or silt loam soil textures (Table 3).

Total roots and root weight are influenced by soil texture. Sandy soil texture promotes the production of more legume plant roots compared to soils with other textures,

consequently impacting root weight. Silt loam content produced fewer roots, although the number is nearly equivalent to that in clay soil; however, the weight of the roots is higher than that in clay soil. It has been demonstrated that sandy soil possesses higher macro-pore content than clay soil. However, organic soil also has high macro-pore content, along with optimal macro pores.

Discussion

The results of the exploration indicate that legume leaflet shapes, such as those found in *Mimosa* with a smooth contour, result in identical nodules that are flatter and smoother. The leaves of *Mimosa* plants have an elongated, lancet shape with a pointed tip and a rounded base. The edges of the leaves are flat, and both the top and bottom surfaces are smooth, with a length ranging from 6–16 mm and a width of 1–3 mm (Ferguson *et al.* 2020). If legume leaflets are more rounded, they tend to produce nodules with a more rounded shape (Feitoza *et al.* 2018). Owolabi and Adedeji (2016) explained that *Calopogonium* leaves have a round (orbicularis) shape, trifoliolate and are dark green with a hairy leaf surface. They have a leaf width of 5.3 cm, petioles length of 1.8 cm, leaf surface length of 4.7–8 cm and leaf thickness of 0.2–2.0 cm, *Vigna unguiculata* leaves are compound with three leaves. The shapes of the leaves vary from oval to egg-shaped and the color is consistent being light green for new plants and dark green for those ready to harvest. Cowpea leaves consist of three leaflets (trifoliolate) arranged alternately. The leaves are green, oval (ovate) or lanceolate, with a length ranging from 6.5–16 cm and a width of 4–10 cm, and a leaf stalk (petiole) length between 5–15 cm. The leaf shape is oval, and if the ratio is 3–5:1, the leaves are lanceolate. The lanceolate leaf shape of cowpeas is dominant over the oval leaf shape, and inheritance is controlled by a single dominant gene. Further studies are needed to identify other types of legumes (Fig. 2). The shapes of various types of root nodules and other legume plants (Fig. 3).

Legumes and some plant species in the monophyletic nitrogen-fixing group produce root nodules that function as symbiotic organs, establishing mutualistic relationships with nitrogen-fixing bacteria. The mode of nodule organogenesis differs from that of lateral root development and also varies among the different types of nodules formed on leguminous and actinorhizal plants. The evolution of new organs is thought to occur through molecular network rearrangements punctuated by specific neo-functional factors. Accumulating evidence suggests that root nodule organogenesis involves the root or lateral developmental pathway. This review describes the current knowledge about the factors/pathways that the ancestors of the nitrogen-fixing clade acquired to control nodule organogenesis (Silva *et al.* 2023).

Root nodules are abnormal tissue parts of plant roots formed due to the interaction between plant roots and *Rhizobium* bacteria supporting the uptake of nitrogen from

the atmosphere (Fitriani *et al.* 2020). Root nodules on domestic plants, especially *Arachis hypogaea*, can reach 56 nodules with effective root nodules of 94.52%. Khurshid *et al.* (2022) have proven that *Arachis hypogaea* plants have 42 nodules in untreated soil and reach 115 nodules if given an application of cow manure equivalent to 15 kg P ha⁻¹ with 74% effective root nodules. Millar *et al.* (2023) demonstrates that differences in the regulation of root nodule symbiosis are evident between domesticated and wild chickpeas and soybeans, but not in lentils, peas and peanuts. This indicates that disruption of mutualism decrease the magnitude of the mutually symbiotic interaction—is a possible consequence of domestication but not a necessary one.

Vigna unguiculata has quite a lot of root nodules reaching 228.53 with effective root nodules of 96.56%. *Mimosa pudica* reaching 143.42, then *Crotalaria incana* with 125.5, *Glycine max* reaching 51, *Arachis hypogaea* reaching 56.33, followed by *Leucaena leucocephala*, *Macroptilium atropurpureum* and *Calopogonium mucunoides* (Table 1; Fig. 4). Zheng *et al.* (2020) demonstrated through principal coordinate analysis and ANOSIM tests that *G. soja* and *G. max* exhibited similar nodule bacterial communities, distinct from the bacterial community of *S. cannabina*. Wild legumes *S. cannabina* and *G. soja* showed higher counts of rhizobia, while *G. max* had a higher abundance of non-rhizobia bacteria. These differences can be attributed to their adaptability to salt-alkaline stress, shedding light on the relative importance of endophytic nodules in relation to culturable *Rhizobium*. Data showed variation in the presence of naturally occurring cowpea-nodulating rhizobia, ranging from poorly to highly effective. There is a prevalence of effective isolates in diverse agroecological zones of Ethiopia where cowpea is cultivated. Further studies are warranted under varied conditions to explore the promising isolates' competitiveness, persistence and potential (Kebede *et al.* 2020).

This study provides fundamental new insights into the effects of Ni on N metabolism, and nodulation may help increase cowpea yield. Given the increase in population and demand for staple foods these results contribute to improved agricultural techniques that increase crop productivity and help maintain human food security (Mendes *et al.* 2023). The nodulation profile of these plants under stress conditions is being studied. The non-heme iron in spinach was then analyzed. This supplement should be combined with fortified soy yogurt. Soy yogurt enriched with heme iron has higher bioavailability than soy yogurt enriched with non-heme iron (Kanimozhi and Sukumar 2023). Tapia-García *et al.* (2020) demonstrated that the most frequently identified plant growth-promoting (PGP) activity among strains isolated from wild legumes is indole-3-acetic acid (IAA) synthesis. Two bacteria *Stenotrophomonas* sp. and *Rhizobium* sp., synthesized at rates exceeding 250 µg/mL, higher than *Azospirillum brasilense* Sp7 (59.77 µg/mL).

Nitrogen fixation and the production of antimicrobial compounds were uncommon, but siderophore production was common among all strains. This study reveals that a variety of nodule-associated bacteria (NAB) with PGP activity are highly prevalent in legume nodules from south-central Mexico.

The plant growth phase greatly influences the number of root nodules in legume plants. In general, root nodules are the highest in the primordial flower phase. Furthermore, the number of root nodules will decrease during the pod formation phase (Table 2). However, the occurrence of nodule formation in plants is also very much determined by the plant species. In line with what has been explained by Nur (2015), sengeron root nodules develop and increase in number in a zigzag graph according to the increasing age of the *Paraserianthes falcataria* (L.) Nielsen sengeron plant, which starts at the age of 2 weeks after sowing. Talams (1988) explained that the number of bacteria in the root nodules of *Glycine max* plants ranged from 13–31.25 × 10⁹ cfu/g. Furthermore, Ro *et al.* (2023) proved that soybeans planted in inceptisol produced effective root nodules ranging from 12–18, with soybean yields reaching 1.1 tons ha⁻¹. Bakari *et al.* (2020) proved that 16–45 soybean root nodules in Ultisol soil were able to produce a dry weight of soybean seeds reaching up to 2 tons ha⁻¹. Rapialdi *et al.* (2022) has demonstrated that the number of soybean root nodules ranges from 16 to 52 to achieve a dry seed weight of 3.17 tons ha⁻¹. Crude protein (CP) levels are high, reaching 20.81% at the flower primordial stage. The high CP content makes the soybean plant very good as quality forage for livestock. In addition Anago *et al.* (2023) added that the nutrient levels of N, P, K, Ca, Mg and Zn in legume leaves remain determinants of seed productivity.

In general, the soil in Padang City in these three sub-districts has a pH < 5.5, but this does not reduce the ability of bacteria to form root nodules in legume plants. It was even found that legume plants were able to form nodules in soil with a pH of 3.32. Soil chemical reactions pH > 5 even produce a decreased number of root nodules compared to the number of legume root nodules at pH 4.7 (Table 5 and equation 2). In fact if you refer to (Solomon *et al.* 2022). *Rhizobium* bacteria in soybean plants of various varieties cannot live at a pH < 4.3. However, during the exploration activities, a soil pH of 3.32 was still found with a total of 18 root nodules on the legume plant *Calopogonium mucunoides* (Clover crop), while on the soybean plant, a total of 83.95 nodules were found at a pH of 4.82. This has also been reported by Shah *et al.* (2021), from soil pH of 5.10 to 5.33, the number of soybean root nodules decreased from 44 to 18.33 in limed soil, while unlimed soil with a soil pH of 5.53 produced root nodules of 16.33 fruit. Then when the soil pH was lower, reaching 5.07, the number of soybean root nodules reached 41.33 fruit. Increasing soil pH increased the number of root nodules to 4.8 and decreased the number of root nodules if the pH increased to 5.43 with a coefficient of determination R² = 0.89.

Furthermore, soil pH does not affect effective root nodules (%) with a value of $R^2 = 0.205$ (Table 6 and equation 3). It is suspected that effective root nodule formation is more influenced by the growth of the host plant, especially the host's metabolism. Healthy growth of the host plant will produce effective root nodules, which are characterized by producing *leghemoglobin*. Basbuga *et al.* (2021) explained that the superiority of legume plants which produce high numbers of nodules was determined through PCA analyses of rhizobial isolates. These isolates were tested and found to be highly resistant to stress conditions, indicating that pH and salt concentrations, especially, had a significant effect on these bacteria.

Soil texture influences the number and weight of legume roots (Table 4 equation 4). The highest number of legume plant roots was found in sandy loam soil, followed by clay texture, and finally, in Silt loam. The highest fresh weight of legume roots was also found in sandy loam texture, followed by silt loam, and finally, in clay soil. A coarser texture produces a higher number of root nodules compared to other soil textures. Effective root nodules are also influenced by soil texture. Soil with a clay texture still produces the lowest effective root nodules compared to sandy or silt loam soil textures (Table 3 and 7). Diatta *et al.* (2020) reported the results of their research, indicating that Mungbean dry matter yield, $\delta^{15}\text{N}$ values, shoot content, amounts of N-fixed and soil N uptake were all higher on the silt loam soil compared to the loamy sand soil. The development of root nodule formation is also greatly influenced by soils. Zheng *et al.* (2020) explained that the ability of clay loam to supply N was weak during the plant growth period. Themba (2021) suggested that Leptsol soil is capable of producing cowpea root nodules compared to other types of soil such as Luvisols, Ferralsols and Fluvisols. Leptsols are typically shallow soils with limited development, often found in areas with steep topography or rock near the ground surface which inhibits the development of deeper soil horizons. Leptsols tend to have little or no soil horizon formed. Leptsols are shallow soils with limited development, often characterized by a lack of significant soil horizons or distinct features of soil formation. They are typically found in areas with rocky or steep terrain where soil development is hindered.

In sandy soil there are more roots than in clay and silt loam soil because sandy soil has larger pore spaces, which allow for better root penetration and growth. This results in a higher root density in sandy soil compared to clay and silt loam soils. This is because silt loam has fewer voids which make it more difficult for the *Rhizobium* bacteria to access the plant roots and form nodules. The soil texture greatly influences bulk density (BD) or soil looseness. The dense soil with a BD value $> 1 \text{ g cm}^{-3}$ will result in limited pore space. In general soils that have a clay texture are often found in mineral soils belonging to the Ultisol, Alfisol and Oxisol orders. Mariati *et al.* (2022) explained that Padang City Ultisol in Koto Tangah, such as Lubuk Minturun

Village, has a BD value of 1.04 g cm^{-3} . For some inceptisols the rough texture dominates with a bulk density value of 0.9 g cm^{-3} . Likewise, Ultisol Lubuk Minturun, Koto Tangah District, had a BD of around 1.21 g cm^{-3} , with a total pore space of 46.51%.

Sandy loam texture will produce loose soil because it is dominated by macro pores or drainage pores. This pore plays a role in controlling water, thereby increasing root development and root respiration. Roots that experience good respiration will also carry out good metabolism. Legume plants that experience good root respiration will be able to increase the number of root nodules. Soils with higher clay content are found in clay textures. The picture shows that clay texture produces fewer nodules compared to sandy soil. This was also explained by Boguta and Sokołowska (2012) that sandy soil's ability to hold water and bind nutrients is low. Good aeration and sufficient macro pores in sandy soil support the development of plant roots and support respiration carried out by the roots so that they can support plant growth. Meanwhile, clay soil has low porosity because it has many micro pores and does not support root growth. So, plants that grow in soil with high clay content are less productive. Likewise, based on Table 7 and equation 5, root weight and pH are positively correlated with the number of root nodules. Similarly, Zavalin *et al.* (2019) indicates that legumes generally thrive in neutral to slightly acidic conditions, with optimal nodule formation occurring around pH 6.5 to 7.0. The limitation of this study is that it has not yet explored the effect of sodium (Na), iron (Fe) and aluminum (Al) content in the soil on the development of legume root nodules. Additionally, a larger sampling area and a greater number of legume plant samples are needed to provide more representative information for the entire observation area.

Conclusion

Sandy loam textured soil produced more nodules than other soil textures. The highest number of nodules was found in the primordial phase of the flower. A soil pH close to 5 resulted in more nodules than lower or higher soil pH levels. The formation of effective root nodules decreased as soil pH increased beyond a certain level. Sandy clay soil is conducive to higher nodule formation followed by clay texture and silt loam clay. In crux, sandy soil had the potential for up to twice the nodule formation compared to clay soil and six times more than organic soil. Despite the presence of abundant organic matter, organic soil was not capable of producing more nodules than sandy or clay-textured soil.

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Author Contributions

J and S planned the experiments and managed the article write up, PN and NY explored the objects, MZHU and WH supervised the experimental work in Laboratories and helped in article formatting, SR facilitated in data analysis.

Conflicts of Interest

The authors declare that there is no conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable to this paper.

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