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Growth and Nodulation Behavior of Three Leguminous Weeds of Mizoram with Potential Utility as Green Manure Crop

JYOTI JOPIR* AND KALIDAS UPADHYAYA
Department of Forestry, School of Earth Sciences and Natural Resources Management, Mizoram University, Aizawl, Mizoram, India
E-mail: jyotijopir10@gmail.com; kumzu70@gmail.com
***Author of correspondence**

ABSTRACT
Legumes contribute substantially to global crop production and soil nitrogen fertility and play a crucial role in agriculture, agroforestry and the ecosystem due to their ability to fix the atmospheric nitrogen. This study investigated the growth and nodulation behavior of three prevalent leguminous weeds viz., *Crotolaria micans*, *Calopogonium mucunoides* and *Aeschynomene indica* in Mizoram. Seeds of the legume species were collected and pretreated and sown in polybags of 13 X 13 cm in March 2020. Observations on the plant height and biomass; the number and biomass of the nodules on different categories of the roots were carried out three months after sowing. The total number of nodules/plants was in the order *A. indica* (488.30) > *C. micans* (21.00) > *C. mucunoides* (12.10). The shape of their nodules was globose, fan shaped and globose with white streak. Nodule biomass and nitrogen content varied across species, with *C. micans* demonstrating superior nitrogen content despite fewer numbers of nodules. These findings shed light on leguminous weed growth and nodulation behaviors, facilitating sustainable agricultural practices.

Key words: Leguminous weed, root nodules, nitrogen content, biomass and nitrogen fixation.

INTRODUCTION

Legumes or Fabaceae are a significant source of Nitrogen (Quilbe et al. 2021) and have been utilized to sustain soil N fertility (Fauci and Dick 1994). In agroecosystems, legumes play a significant role due to their capacity to create a symbiotic relationship with soil bacteria (rhizobia) that helps in forming the root nodules which in turn fix the atmospheric nitrogen (Mortier et al. 2012, Abd-Alla et al. 2014), a significant contributor to the global nitrogen cycle. Generally, biological nitrogen fixation by legumes serves as the primary nitrogen supply for crop rotation in sustainable and organic agricultural systems. Besides, many leguminous herbs are being utilized as green manure crops for boosting agricultural productivity. Apart from agricultural legumes, nodulated wild (herb and tree) legumes have the ability to fix nitrogen, promote reforestation, and reduce soil erosion (Ahmad et al. 1984).

Nodulation is a potent mechanism that boosts the legume's competitiveness by increasing their ability to obtain nitrogen (Ferguson and Gresshoff 2015). A species' nodulation and nitrogen-fixing ability are significantly governed by climatic conditions (Gibson and Jordan 1983). Major factors that contribute to nodulation failure include high temperatures and a lack of moisture, which have an impact on all phases of the symbiosis and limit rhizobial development and survival in soil (Hungria and Vargas 2000). Soil factors such as salt stress, high temperature, low water content, high or low soil pH, nutrient insufficiency and pesticide application have a significant impact on nodule formation and nitrogen fixation in legumes (Abd-Alla et al. 2016). Over the past decades numerous studies have examined nodule organogenesis at the molecular, cellular and organ sizes (Stacey et al. 2006, Stougaard 2000, Crespi and Galvez 2000) however, little knowledge has been gathered on the nodule number and biomass dynamics over the course of the growth cycle (Voisin et al. 2010). Estimating nodules during various developmental phases of a species can aid with fertilizer application timing, especially since the size and number of nodules are directly related to atmospheric nitrogen fixation (Kashyab et al. 2012). Legume cultivation promotion and crop engineering to improve nitrogen fixation will reduce the need for artificial nitrogen fertilizers and contribute to both short- and long-term objectives for more sustainably farmed agriculture (Charpentier and Oldroyd 2010). There are numerous leguminous weeds growing abundantly in degraded lands and roadside of Mizoram and these weeds can be used as green manure crops for improving soil fertility in croplands. Understanding their growth and nodulation potential is necessary for utilizing them as source of soil amendment for better crop production. Hence, an attempt was made to investigate the growth and nodulation behavior of three abundantly found leguminous weeds of Mizoram.

MATERIALS AND METHODS

Study area

Mizoram (21°56'N - 24°31'N and 92°16'E to 93°26'E) covers an area of 21,081 km^2 and is one of the seven sister states of north-east India (Fig. 1). The state shares an inter-state border with Assam, Tripura and Manipur and also shares an international border with Myanmar and Bangladesh. The Tropic of Cancer passes through the states. Aizawl is the capital of the state and is 1132 m asl that lies between 21^{o}58'- 21^{o}85' N and 90^{o}30'- 90^{o}60' E). It is located north of the Tropic of Cancer in the northern part of the state. The area experiences a monsoonic type of climate.

A polypot experiment was carried out during the month of March-May of 2020 in the Department of Forestry, Mizoram University, Aizawl, India, to study the growth, biomass accumulation and nodulation pattern of leguminous weeds viz., *Calopogonium mucunoides* Desv. *Aeschynomene indica* L. and *Crotolaria micans* Link.

Methodology

Seeds of the selected weed species were collected from plants growing naturally in the wild and roadside areas. The seeds of the *C. micans* and *C. mucunoides* were soaked in cold water for 24 hours before sowing whereas the seeds of *A. indica* were treated with 95% H_2SO_4 for 5 minutes. The seeds were sown in polypots of 13 X 13 cm size in March 2020. The soil to be filled in the polythene bags was collected from the forest and mixed with sand and FYM in a 3:1:1 ratio. The characteristics of the soil

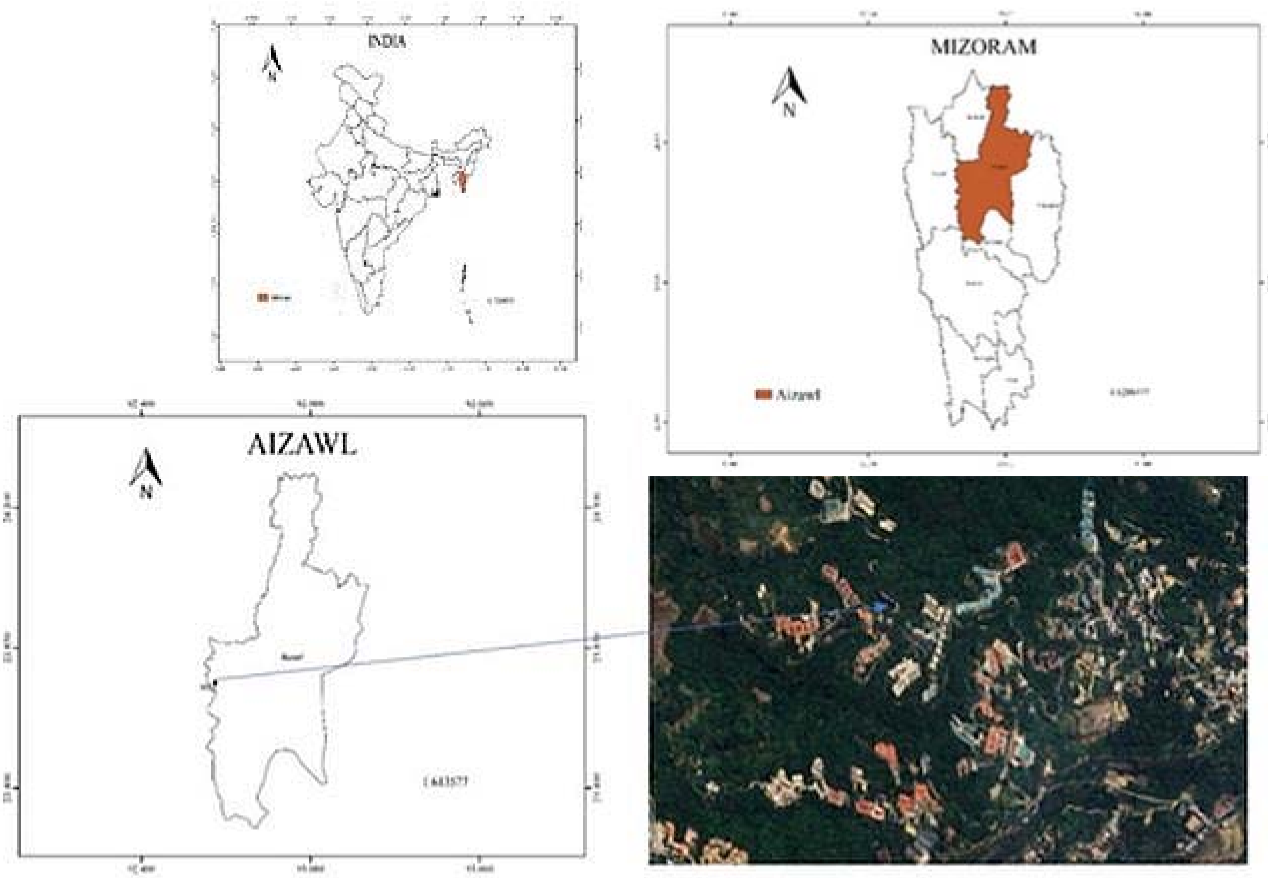

Figure 1. Study area

potting mixture are shown in Table 1.

Table 1. Soil parameters used for filling the polybags

Parameter	Mean ± Standard error
pH	5.1 ± 0.08
SOC %	1.93 ± 0.04
Available N (Kg/ha)	343.02 ±1.64
Available P (Kg/ha)	273.48 ±4.76
Available K (Kg/ha)	86.59 ±2.16
WHC %	33.38 ±0.54

The experiment was laid out in a Completely Randomized design with 10 replications for each species. The replicated plants of each species for growth and nodulation behavior were observed after three months of sowing. The plants were removed from the polypots along with the soil to a big container. Nodules were washed in running water and separated from the roots and nodules from primary, secondary and tertiary root systems were collected separately. The fresh and dry weight of the shoot and root and the nodules from different root systems were recorded separately. The nodules from different root categories were pooled together to determine the nitrogen content. Nitrogen (N) contents from roots, shoots and nodules were determined by CHNS Analyzer (Perkin Elmer 2400 Series 2).

Analysis of variance was done for the data recorded on the nodules produced, the weight of the nodules, plant height, shoot and root biomass and also the nitrogen content of the various parts of the plants and the mean obtained was subjected to Duncan's Multiple Range test (Gomez and Gomez 1984).

RESULTS AND DISCUSSION

Growth and biomass

After 3 months it was observed that the shoot and root length of the leguminous species varied significantly ($p < 0.05$) with shoot and root length ranged from 45.70 to 102.51 cm and 19.10 to 51.74 cm, respectively. While *A. indica* have the highest values for the shoot and root length (Table 2) both *C. micans* and *C. mucunoides* showed no differences in their values. For the fresh and dry biomass of shoot, value ranged from 15.52 to 46.91 g and 3.25 to 11.95 g, respectively (Table 2). The fresh and dry biomass of the roots for all the species were almost similar. The variation in plant height and the biomass during the growth period was most likely caused by the legume's distinct genetic makeup.

Number of nodules produced by the leguminous weed species

The present study showed that nodule formation was significantly ($p < 0.05$) higher in *A. indica* followed by *C. micans* and the lowest by *C. mucunoides* (Table 3). However, secondary roots showed the most nodulation for all the species followed by tertiary and primary roots for *C. micans* and *C. mucunoides*. However, the number of nodules produced in the tertiary roots of *A. indica* was lower than in the primary roots. Variation in the number of nodules might be due to the genetic variation among the species. The genetic behavior of different species as well as the environment have an impact on root nodulation (Gibson and Jordon 1983, Pokhriyal et al. 1991) or may be related to the bacteria and plant species adaptability (Kucuck and Cevheri 2014). A similar study reported that *Mimosa* species produced

Table 2. Shoot and root length and the fresh and dry biomass of the shoot and root of leguminous species (n= 10)

	Plant height (cm)		Fresh biomass (g)		Dry biomass (g)	
Legume species	Shoot	Root	Shoot	Root	Shoot	Root
C. micans	45.70^{c}	19.10^{b}	17.88^{b}	1.83^{a}	4.26^{b}	0.78^{a}
C. mucunoides	73.43^{b}	28.16^{b}	15.52^{b}	1.92^{a}	3.25^{b}	0.89^{a}
A. indica	102.51^{a}	51.74^{a}	46.91^{a}	1.84^{a}	11.95^{a}	0.83^{a}

*Values with the same letter along a column do not differ by Duncan's test ($p < 0.05$).

Table 3. Number of nodules/plants produced on different root categories of the three leguminous weed species (n = 10)

Legume Species	Primary root	Secondary root	Tertiary root	Total root nodules
C. micans	2.80[b]	3.50[b]	14.7[b]	21.00[b]
C. mucunoides	0.40[b]	1.40[b]	10.30[b]	12.10[b]
A. indica	188.10[a]	256.10[a]	40.10[a]	484.30[a]

Values with the same letter along a column do not differ by Duncan's test ($p < 0.05$)

several nodules that ranged from 0 to 120.6 after 4 months of harvest (de Castro Piers et al. 2018) which was lower than our findings. The inadequate nodulation found in *C. mucunoides* as compared to the other two legumes can be attributed to a combination of environmental factors.

The highest level of nodulation/plant in *A. indica* was recorded in the secondary roots (256.10) followed by primary (188.10) and tertiary roots (40.10). Kashyap et al. (2012) also observed a similar trend in the nodule production and growth among three nitrogen-fixing tree species viz. *Alnus nitida*, *Acacia catechu* and *Albizia chinensis*. However, in the case of *C. micans* and *C. mucunoides*, maximum number of nodules was observed in the tertiary roots followed by secondary and primary roots (Table 3). Irin and Biswas (2021) observed maximum nodulation after 30 days of sowing in *S. rostrata* (36.53/plant) and the lowest by *L. leucocephala* (11.00/plant). Pramanik et al. (2009) reported maximum nodules plant^{-1} was produced by *S. aculeata* (21.99), *C. juncea* (17.69) and *P. mungo* (17.44) after 30 days of sowing. Overall, the number of nodules was at par between *C. micans* and *C. mucunoides* irrespective of the root categories (Table 3).

All the three species showed different shapes of nodules (Fig. 2). While the nodules of *C. micans* were fan-shaped with pinkish-white in color, nodules of *C. mucunoides* were globose and brownish in color with white streaks on the surface, and in *A. indica* they were brown globose.

Fresh and dry biomass of root nodules produced by the leguminous weed species

Fresh and dry biomass of the nodules was significantly different ($p < 0.05$) for all the species. *A. indica* recorded the highest fresh and dry biomass of nodules in the primary (132.72 and 61.78 mg, respectively) and secondary root systems (179.51 and 82.98 mg, respectively). However, *C. micans* showed the highest fresh and dry biomass of nodules on tertiary roots (97.55 and 26.76 mg, respectively) which may be attributed to the nodule size. The

Table 4. Fresh and dry biomass of the nodules (mg/plant) produced on different root categories of the three leguminous weed species (n= 10)

	Fresh biomass of the nodules			
Legume species	**Primary roots**	**Secondary roots**	**Tertiary roots**	**Total**
C. micans	19.09[b]	31.66[b]	97.55[a]	148.31[c]
C. mucunoides	1.67[b]	3.32[b]	9.13[b]	14.12[b]
A. indica	132.71[a]	179.51[a]	22.95[b]	484.30[a]
	Dry biomass of the nodules			
	Primary roots	**Secondary roots**	**Tertiary roots**	**Total**
C. micans	5.36[b]	7.87[b]	26.76[a]	39.99[b]
C. mucunoides	0.61[b]	1.48[b]	3.61[b]	5.70[b]
A. indica	61.78[a]	82.98[a]	8.52[b]	153.28[a]

Values with the same letter along the column do not differ by Duncan's test ($p < 0.05$)

Figure 2. Nodule formation by different leguminous weed species.a) *Aeschynomene indica*, b) *Calopogonium mucunoides* and c) *Crotolaria micans*

biomass of the nodules was found to be proportional to the number of nodules found on the primary, secondary and tertiary roots.

Nitrogen content

The total nitrogen concentration of the roots, shoots, and nodules varied significantly across the species under study (Table 5). Maximum nitrogen content in the shoot was in *C. micans* (1.53%) and minimum in *A. indica* (1.12%). While *C. mucunoides* recorded the highest nitrogen content (1.17%) in the root, *A. indica* recorded the lowest (0.98%). *C. micans* (2.31%) had the maximum nitrogen content in the nodules followed by *A. indica* (1.82%) and *C. mucunoides* (1.58%).

Higher nitrogen content both in the shoots and roots of *C. micans* and *C. mucunoides,* respectively, could be attributed to the plants improved nitrogen dynamics (Beniwal et al. 1995). Maximum nitrogen content in the nodules was observed by *C. micans* although the number of nodules and nodule fresh biomass was much lesser than *A. indica* (Tables 3 and 4). Similar relationship where nodules of *Dalbergia sericea* showed higher nitrogen content but the fresh biomass produced was lower as compared to *Albizia chinensis* and *Dalbergia sissoo* was reported by Purohit et al. (1997). Kashyab et al. (2012) also observed a similar relationship between nodule biomass and nitrogen content in *Albizia chinensis*, *Acacia catechu* and *Alnus nitida*.

Table 5. Nitrogen content (%) present in different parts of the leguminous species (n= 3)

Legume species	Shoot	Root	Nodules
C. micans	1.53[a]	1.09[b]	2.31[a]
C. mucunoides	1.29[b]	1.17[a]	1.58[c]
A. indica	1.12[c]	0.98[c]	1.82[b]

Values with the same letter along a column do not differ by Duncan's test ($p < 0.05$).

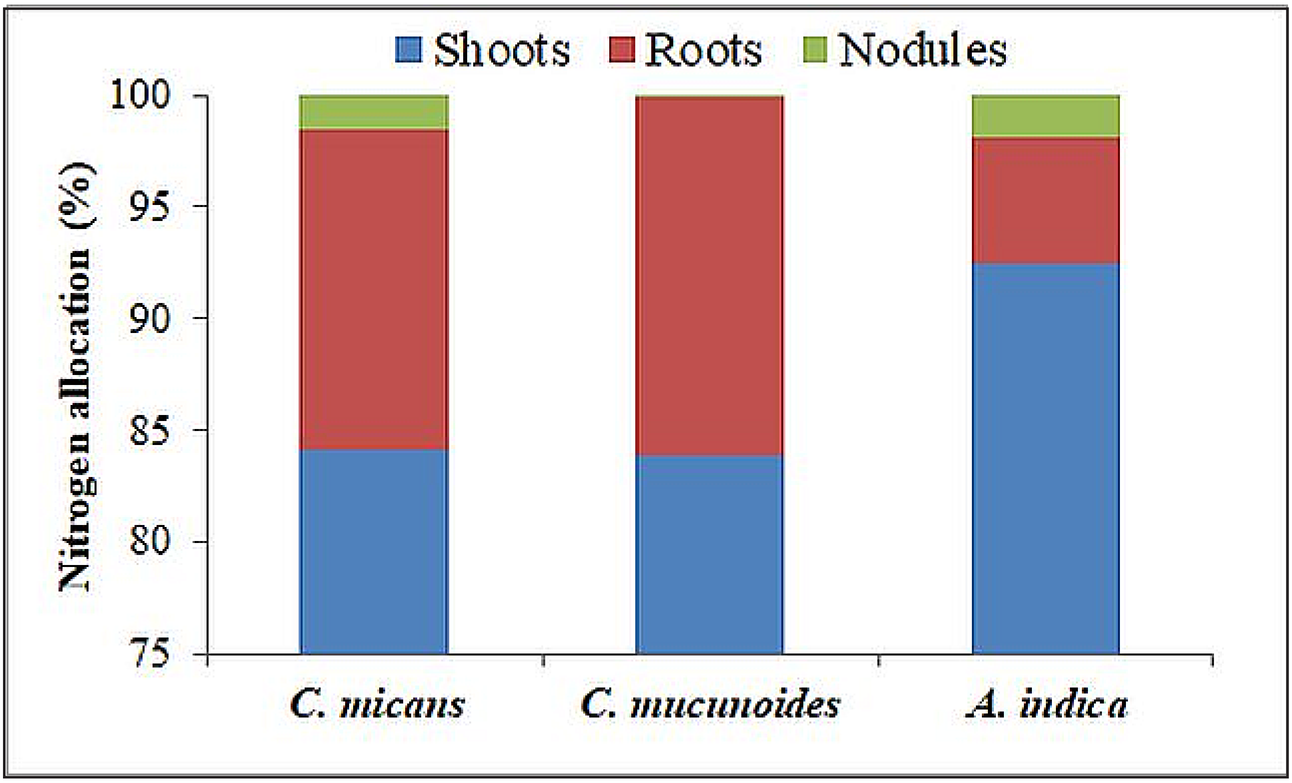

Figure 3. Nitrogen allocation (% of the shoots, roots and nodules) of different leguminous weed species

Nitrogen allocation (irrespective of the nitrogen content) in shoots, roots and nodule biomass varies among the species (Fig. 3). The maximum nitrogen allocation was recorded from the shoot biomass for all the studied species. Nitrogen distribution from the root biomass was 14.31% for *C. micans*, 15.96% for *C. mucunoides* and 5.63% for *A. indica*. However, nitrogen allocation from the nodule biomass (irrespective of different root categories) for all the species was very low.

CONCLUSIONS

The study explored the nodulation behavior of three leguminous weed species in Mizoram. *A. indica* exhibited significant nodulation, especially in the secondary roots. The Nitrogen content varied in shoots, roots, and nodules across the species with *C. micans* having the highest nitrogen content in its shoot and root nodules. These findings enhance our understanding of legume nodulation dynamics, offering insights into species-specific responses and the potential for sustainable agriculture. However, a detailed investigation of field conditions should be carried out to understand the relationship between the nodulation and symbiotic nitrogen-fixing ability of the studied species to ascertain their suitability as green manure crops.

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Conflict of interest: Authors declare no conflict of interest.

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