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Root Nodule Categorization and Their Relation with Plant Growth in Peanut Crop Grown in Alfisols

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Abstract

To investigate the relationship between nodule category and plant growth characteristics in peanut, nodules were classified into three categories: large size nodule (LSN) (>2 mm), medium size nodule (MSN) (1-2 mm) and small size nodule (SSN) (<1 mm) and their position on roots (primary and lateral roots). The number of LSNs on primary roots was found to be much higher than on lateral roots. The number of MSN developed on primary and lateral roots was comparable, whereas, lateral roots had a higher SSN number. In addition, when comparing the LSN and MSN numbers to SSN number, the plant growth parameters showed a high positive correlation. Multivariate regression analysis yielded similar results. This nodule classification technique aids in the identification of peanut nodules and their role in quantification of plant growth characteristics. Further, this technique can be used in identifying/screening of efficient nodulating peanut genotypes.

Key words: Categorization, Nodules, Peanut, Plant growth attributes.

Sustainable legume production can be achieved by maximizing the efficiency of symbiotic nitrogen fixation, the principal source of nitrogen and a necessary mechanism for plant growth. The nitrogen fixation process is critical in peanut (*Arachis hypogaea* L.) since it provides 50-80% of the total crop nitrogen requirement and the rate of nitrogen-fixation activity is determined by the quantity and developmental stages of root nodules (Nievas *et al.,* 2012). Furthermore, unlike other legumes, peanuts have a unique nodule development process, with the bulk of root nodules forming on the first order roots at the tap root's basal region (Bogino *et al.,* 2011). The majority of peanut root nodules are found on the first order roots near the basal region (top 8 cm) of the tap root (Tajima *et al.,* 2006). Peanut generally lacks root hairs on the tap root because the surface cell layers are shed, so nodules form at the base of lateral roots where rosette-type root hairs upto 4 mm in length are found.

The ability of the entire root system to fix nitrogen was found to be linked to leghaemoglobin content, which is influenced by nodule size. To properly understand the specific relationship between nodule size and plant growth in peanut, more research on nodule size and distribution pattern in the root system is required. The study of nodule categorization based on indeterminate, determinate, early infection and late symbiotic parameters has been extensive. Peanut nodules, on the other hand are usually ambiguous and there is a lot of variation in nodule form, quantity and position on the root, which may have impact on plant growth. So far, nodule count and nodule weight have been used to measure variation in nodule production at the field level. Nodule classification based on size, weight and position on roots may aid in better nodule profiling and understanding of their role in peanut plant growth.

There is currently a scarcity of data on the association between nodule size categories and growth characteristics in semi-spreading peanut type plants. Hence, the current study was carried out to characterize peanut nodules in terms of size, weight and their position on roots as well as to examine the impact of nodule size categories on dry matter production of various plant parts. In addition, a quantitative relation (mathematical model) was estimated between nodule size fractions and plant growth characteristics.

The field experiment plot was prepared by deep ploughing and harrowing with a mini-power tiller. Peanut cultivar var. K1812 (semi-spreading type) was grown in 30 × 30 m^2 area. On November 20, the crop was planted with 30 cm spacing between rows and 15 cm between plants inside each row. The crop was fertilized with a basal application of 30 kg N, 50 kg P_2O_5 and 60 kg K_2O/ha. External rhizobium application was not done to achieve natural variation in nodulation. At 25 and 50 days after seeding, two manual hand weedings were performed.

A total of 100 healthy peanut plants (n=100) were gently scooped along with soil adherent to roots using a shovel at 50 per cent flowering. The root nodule system was sampled with extreme caution so that it would not be disturbed. The

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roots were carefully dug out of the soil and cleaned in water. Later, the shoots were cut at ground level and the roots with intact nodules were stored in a polythene packet.

Nodule from primary roots and lateral roots were separated from each plant and placed in polythene packet. The nodules were then divided into three sizes: large-sized nodules (diameter /length >2 mm); medium sized-nodule (1-2 mm) and small-sized nodule (<1 mm). To avoid desiccation, the entire operation was carried out by five skilled individuals and immediately the nodules in different categories were weighed using an electronic analytical weighing balance.

Plant growth characteristics

Plant growth characteristics such as plant height, leaf number, leaf weight, stem weight and root weight were measured using a high efficiency electronic balance for each plant (n=100). The total above ground weight was estimated by summing the dry weights of the leaves and stems. SPSS was used to do Multivariate regression analysis, Pearson correlation and p values were computed as per the standard procedure.

LSN was found to be more developed on primary roots ($\bar{x}$ =10.36) and less developed on lateral roots ($\bar{x}$ = 4.5), but MSN was found to be comparable on primary root ($\bar{x}$ = 10.0) and lateral roots ($\bar{x}$ =8.9). In addition, SSN on lateral roots ($\bar{x}$ =11.3) were higher than on primary roots ($\bar{x}$ 10.4), but they were comparable.

In primary root and lateral roots, the average number of total nodules is 30.8 and 24.4, respectively. Despite the fact that nodule growth was generally independent, it was observed that the LSN on primary roots and MSN on primary roots had a positive association, showing that the primary

Table 1: The Pearson correlation coefficient (r) between nodule number and nodule fresh weight in peanut plants.

Growth	NLSN			NMSN			NSSN			NTN		
attribute	PR	LR	PR+LR	PR	LR	PR+LR	PR	LR	PR+LR	PR	LR	PR+LR
Correlation coefficient (r)												
PH	0.73	0.48	0.70	0.44	0.37	0.47	0.47	0.53	0.61	0.65	0.62	0.69
LN	0.74	0.51	0.71	0.43	0.38	0.47	0.50	0.56	0.62	0.66	0.63	0.70
LDW	0.62	0.34	0.57	0.35	0.29	0.37	0.40	0.53	0.55	0.55	0.52	0.58
SDW	0.64	0.40	0.60	0.36	0.36	0.41	0.35	0.51	0.50	0.54	0.52	0.58
TADW	0.64	0.38	0.60	0.36	0.33	0.40	0.38	0.53	0.53	0.55	0.55	0.60
RDW	0.65	0.43	0.62	0.39	0.42	0.47	0.37	0.52	0.53	0.57	0.59	0.63
P value												
PH	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
LN	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
LDW	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000
SDW	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
TADW	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
RDW	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Growth	WLSN			WMSN			WSSN			WTN		
attribute	PR	LR	PR+LR	PR	LR	PR+LR	PR	LR	PR+LR	PR	LR	PR+LR
Correlation coefficient (r)												
PH	0.80	0.48	0.77	0.58	0.36	0.60	0.52	0.41	0.52	0.81	0.57	0.79
LN	0.82	0.53	0.80	0.58	0.38	0.61	0.53	0.49	0.59	0.83	0.64	0.83
LDW	0.76	0.44	0.72	0.51	0.33	0.54	0.43	0.40	0.48	0.74	0.53	0.73
SDW	0.77	0.50	0.75	0.56	0.42	0.62	0.42	0.37	0.45	0.76	0.56	0.75
TADW	0.78	0.48	0.75	0.54	0.39	0.59	0.43	0.39	0.47	0.77	0.56	0.75
RDW	0.77	0.46	0.74	0.54	0.41	0.60	0.41	0.29	0.38	0.76	0.49	0.72
P value												
PH	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
LN	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
LDW	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
SDW	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
TADW	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
RDW	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000

Bold values indicate significant difference at the p<0.05 level.

PH: Plant height, LN: Leaf number, LDW: Leaf dry weight, SDW: Shoot dry weight, TADW: Total above ground dry weight, RDS: Root dry weight, LSNN: Large sized nodule number, MSNN: Medium sized nodule number, SSNN: Small size nodule number, NTN: Total nodule number (NLSN+NMSN+NSSN) WLSN: Large-sized nodule weight, WMSN: Medium-sized nodule weight, WSSN: Small-sized nodule weight, NTN: Total nodule weight (WLSN+WMSN+WSSN), PR: Primary root, LR: Lateral root PR+LR both primary and lateral roots.

root produces the majority of LSN and MSN. Furthermore, the number of total nodules on primary root and lateral roots was strongly associated. The weight of LSN on primary roots was 3 times that of lateral roots, while the weight of MSN on primary roots was higher ($\bar{x}$ =0.08) compared to lateral roots ($\bar{x}$ =0.07). On the other hand, the weight of SSN on primary root was lower (0.05) than on the lateral root (0.07) (Table 1).

The weight of MSN and SSN on primary roots was shown to have a positive relationship ($p<0.001$). On lateral roots, there was a positive association between MSN and SSN, although it was not significant (Fig 1). All of the growth parameters had a substantial positive correlation with all of the nodule categories (LSN, MSN and SSN). LSN, MSN and SSN demonstrated positive correlations with plant height (PH), leaf number (LN), leaf dry weight (LDW), stem dry weight (SDW), total above ground biomass dry weight (TADW) and root dry weight (RDW) regardless of their positions on roots. There were also strong correlations between nodule weights and growth parameters. The weights of LSN, MSN and SSN were also found to have substantial correlations with all of the growth parameters in multiple regression analysis (Table2).

Peanut nodules are typically smaller than those of other legume crops, but they have a greater overall potential for N-fixation (Tajima *et al.,* 2007). Furthermore, nodule size has been reported to have a considerable impact on N-fixation activity (Rowland *et al*., 2015) and the nodule size range was similar to what was observed in the current study, where the majority of root nodules were around 2 mm in diameter (LSN and MSN). The significant positive relationship between LSN and MSN could be explained by enhanced N-fixation activity due to increased phloem supply (Purcell *et al.,* 1997). Our observations indicated that root nodule formation was more prevalent on primary roots and the number of nodules on primary roots are far greater than those on lateral roots. Although peanut has a deep root, most of the nodules are formed on the shallow region of the tap root (Ketring and Reid, 1993). The formation of such

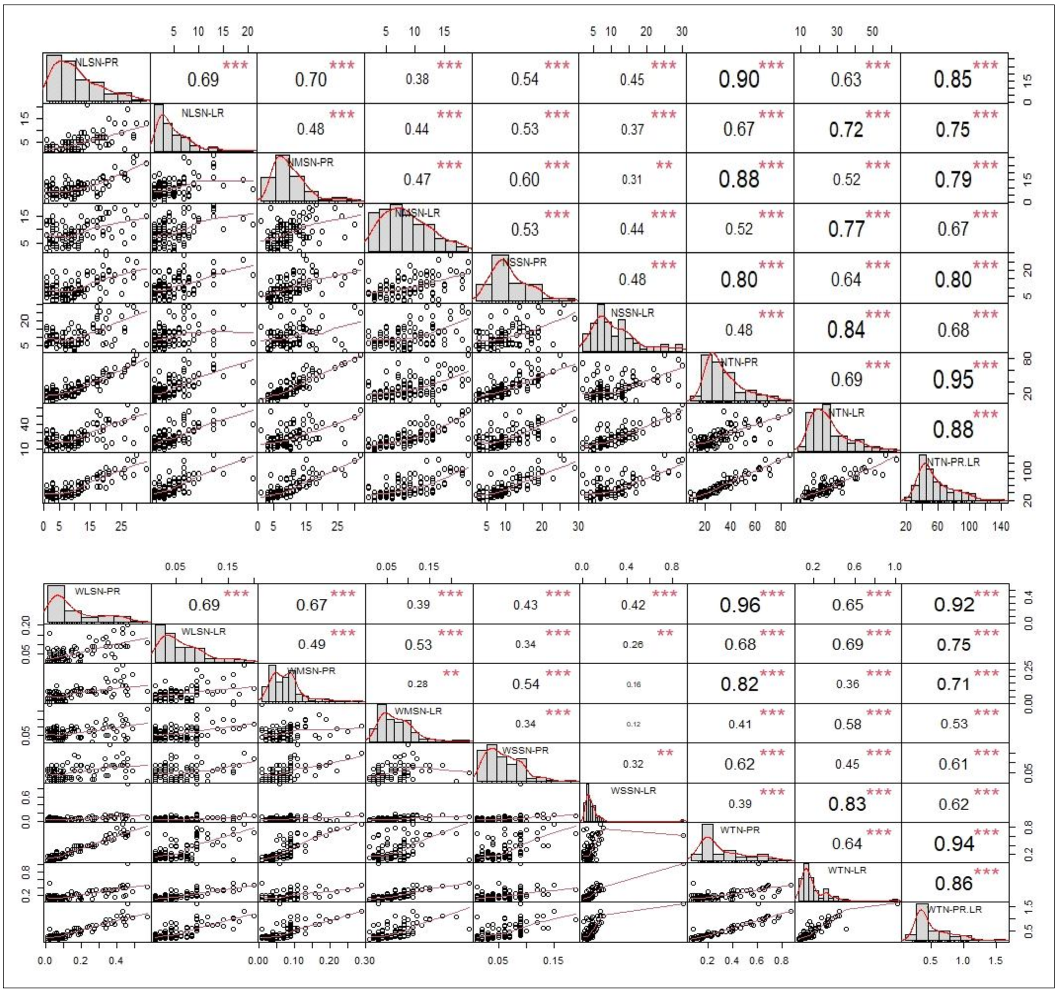

Fig 1: The Pearson's correlation coefficient ® between different nodule categories of peanut (n=100). NLSN: Large-sized nodule number, NMSN: Medium-sized nodule number, NSSN: Small-sized nodule number, NTN: Total nodule number, PR: Primary root, LR: Lateral root, PR.LR both primary and lateral roots, WLSN: Large-sized nodule weight, WMSN: Medium-sized nodule weight, WSSN: Small-sized nodule weight, WTN: Total nodule weight, *significant ($p<0.05$), **significant ($p<0.01$), ***significant ($p<0.001$).

Table 2: Multivariate regression equations showing the association between plant growth characteristics and different nodule size categories in peanut (var. K1812) (n=100).

Regression equation	R^2	Adjusted R^2	P value			
			Regression	LSN	MSN	SSN
PH = 14.1+0.319 NLSN-0.086 NMSN+0.207 NSSN	0.550	0.536	<0.001	<0.001	0.218	<0.001
LN = 121.2+3.645 NLSN-1.133 NMSN+2.390 NSSN	0.759	0.577	<0.001	<0.001	0.125	<0.001
LDW = 1.97+0.052 NLSN-0.024 NMSN+0.046 NSSN	0.405	0.386	<0.001	<0.001	0.148	0.001
SDW = 2.48+0.064 NLSN-0.012 NMSN+0.035 NSSN	0.397	0.378	<0.001	<0.001	0.506	0.019
TADW = 4.45+0.116 NLSN-0.036 NMSN+0.081 NSSN	0.411	0.392	<0.001	<0.001	0.290	0.003
RDW = 0.719+0.043 NLSN-0.00462 NMSN+0.023 NSSN	0.427	0.409	<0.001	<0.001	0.997	0.024
PH = 14.68+20.51 WLSN+9.09 WMSN+10.69 WSSN	0.628	0.617	<0.001	<0.001	0.217	0.008
LN = 124.09+224.87 WLSN+84.69 WMSN+161.05 WSSN	0.697	0.687	<0.001	<0.001	0.242	<0.001
LDW = 2.03+4.19 WLSN+0.90 WMSN+1.94 WSSN	0.546	0.532	<0.001	<0.001	0.586	0.032
SDW = 2.35+4.47 WLSN+3.33 WMSN+1.52 WSSN	0.588	0.576	<0.001	<0.001	0.063	0.116
TADW = 4.38+8.66 WLSN+4.23 WMSN+3.46 WSSN	0.584	0.571	<0.001	<0.001	0.204	0.056
RDW = 0.823+3.39 WLSN+1.96 WMSN+0.22 WSSN	0.559	0.546	<0.001	<0.001	0.126	0.748

Bold values indicate significant difference at the p<0.05 level.

PH plant height, LN leaf number, LDW leaf dry weight, SDW shoot dry weight, TADW total above ground dry weight, RDS root dry weight, LSNN large sized nodule number, MSNN medium sized nodule number, SSNN small size nodule number, WLSN large-sized nodule weight, WMSN medium-sized nodule weight, ASSN small-sized nodule weight.

nodules could be related to the need for more oxygen for nitrogen fixation and nodule activity is often impeded by low oxygen levels (Weisz and Sinclair, 1987). The LSN and MSN numbers associated with primary and lateral roots were more successful in boosting peanut plant growth characteristics, according to the current data. Hence, while evaluating the performance of peanut cultivars, nodule categorization may be used to estimate N-fixation ability, which influences dry matter formation and as a result, higher yields.

CONCLUSION

The nitrogen fixation process in legumes is attributed to the root nodules and is more influenced by the size and position of nodules on the roots. Our results clearly revealed that the growth parameters *viz*., plant height, leaf number, leaf weight, stem weight and root weight were positively associated with nodule size and weight irrespective of their position on the roots. Further, it was noted that primary roots produced majority of large and medium sized nodules, while majority of small nodules were observed on lateral roots. Hence, it can be construed that primary roots are more effective in nitrogen fixation process as compared to lateral roots.

Conflict of interest: None.

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