Distribution Pattern of Root Nodules in Relation to Root Architecture in Two Leading Cultivars of Peanut (*Arachis hypogaea* L.) in Japan

Ryosuke Tajima¹, Shigenori Morita¹ and Jun Abe²

¹Field Production Science Center, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Nishitokyo, Tokyo 188-0002, Japan; ²AE-Bio, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo 113-8657, Japan

Abstract: To effectively utilize symbiotic nitrogen fixation, we examined the formation of root nodules along with root system development in two leading peanut cultivars in Japan, Chibahandachi and Nakateyutaka. Differences in the number, size and distribution pattern of root nodules between the two cultivars are discussed in relation to their root architecture. Many root nodules are formed on the 1st-order lateral roots in the peanut. The difference between the two cultivars in the number of nodules on the 1st-order lateral roots and the diameter of the 1st-order lateral roots at the basal part of the taproot increased during secondary thickening period. Those changes were significantly greater in Chibahandachi than in Nakateyutaka at later growth stages. Chibahandachi had fewer, but larger nodules than those in Nakateyutaka. In Nakateyutaka, a larger number of new nodules were formed on the lateral roots at the middle part of the taproot than in Chibahandachi. This suggests that in Chibahandachi nodules grow for a longer period during plant growth, and in Nakateyutaka new nodules are formed even at late stages of plant growth. In addition, there appears to be an optimal diameter of the 1st-order lateral roots for nodulation at each growth stage.

Key words: *Arachis hypogaea* L., Lateral root, Nodule size, Peanut, Root nodule, Root system.

Sustainable agriculture might be established with the effective utilization of the symbiotic nitrogen fixation by legumes. Nitrogen fixation by leguminous crops varies widely with the species and cultivar as well as with the environmental condition (Phillips, 1999). However, such difference in nitrogen fixation ability was mostly discussed in relation to total number and weight of nodules (Hardarson et al., 1993; Bell et al., 1994; Pazdernik et al., 1997).

The nitrogen-fixing activity is also known to differ depending on their position in the soil and the growth stage of the plant in both soybean (McDermott and Graham, 1989) and common bean (Vikman and Vessey, 1993a, 1993b). Studies on the nodule distribution in relation to root architecture were very limited, though such information is important to understand the nitrogen fixation by the whole root system of a leguminous plant. Kamata (1958) conducted experiments under various conditions and reported that most nodules are formed on the axis of taproots in soybean and peanut, whereas they are formed on the lateral roots in common bean. This study, however, offered only qualitative information from a brief observation.

Moreover, the distribution of nodule size in the root system is also important to determine nitrogen fixation of a leguminous plant, because the nitrogen-fixing activity of individual nodules is affected by the nodule size (King and Purcell, 2001). In general, the distribution of nodule size varies with the plant growth stage. Tamura (1992) tried to describe the distribution of nodule size using Erlan’s model, and Ikeda (2003) used four parameters including the partitioning of photosynthates in the root system to describe the distribution of nodule size in soybean. However, these modeling studies for soybean did not pay attention to the architecture of root system, and the distribution of nodule size is still unknown in other legumes.

Peanut is an important rotational legume crop, especially in arid and semi-arid areas, because it can grow under drought conditions (Reddy et al., 2003). Nodule formation in peanut is known to differ from that of many other legumes. Root nodules develop only at sites of lateral root emergence in peanut, whereas the nodulation often occurs at sites of root hair formation in other legumes (Uhedaka et al., 2001). However, information on nitrogen fixation in peanut is limited compared with that of soybean and common bean (Bell et al., 1994; Khan and Yoshida, 1994; Daimon et al., 1999; Daimon and Yoshikawa, 2001).
The objective of this study was to examine the formation of nodules with reference to root architecture and the distribution of nodule size in two leading peanut cultivars in Japan. Such manual observation for mapping the location and size of root nodules is a primitive but essential step for a detailed analysis of the nitrogen fixation of legumes in the field. Differences in the number, size, and distribution pattern of root nodules between the two cultivars are discussed in relation to their root architecture.

**Materials and Methods**

Two leading peanut cultivars in Japan, Chibahandachi and Nakateyutaka were grown in the field at Field Production Science Center of The University of Tokyo, Tokyo, Japan (lat. 35° 43’ N, long. 139° 32’ E) in 2003 and 2004. The two peanut cultivars are both bush types, and the flowering starts at similar date in both two cultivars. Chibahandachi is a late maturing Virginia-type variety and Nakateyutaka is a medium maturing type derived from a cross between Virginia and Spanish types. The soil was a Kanto loam type (humic andosol) with a topsoil layer (0–35 cm) of dark humic silty loam (pH 6.2) and a subsoil layer (below 35 cm) of red-brown silty clay loam (Yamagishi et al., 2003). Seeds of the two cultivars were inoculated with *Bradyrhizobium* ssp. (J2P21, Tokachi Nokyoren) and planted at a 5cm depth in the field by hand on 26 May in both years. Row direction was east to west with a row spacing of 0.7 m and a plant spacing of 0.3 m. Each plot size was 21 m² (7 m × 3 m), and was arranged in a completely randomized block design with five (2003) or six (2004) replications. Chemical fertilizer including N, P and K at the rate of 15, 50, and 50kg ha⁻¹, respectively was applied as basal dressings before planting. Plots were rainfed and weeded manually throughout the growing period.

Five plants of each cultivar were collected four times: 36, 66, 94, and 139 days after seeding (DAS) in 2003, and 36, 65, 96, and 134 DAS in 2004. At each growth stage, aboveground plant parts were harvested, dried at 80°C for 72 h, and weighed. After the aboveground parts were removed, the soil monolith with roots (0.3 m in width crossing the row including the plant at the center, 0.1 m in length in the direction of the row, and 0.3 m in depth) was taken and the root system was carefully washed out from the soil. The taproot was cut into segments of 2 cm long along the root axis and the diameter of each root segment was measured at the middle of each segment. All the 1st-order lateral roots on each root segment were counted and their length and diameter at 2mm from the base were measured. The number of nodules formed on the root segments of the taproot and all the 1st-order lateral roots were recorded. The size of each nodule was classified into three classes based on their diameter (<1.0, 1.0-2.0 and 2.0-3.0 mm).

**Results**

The two cultivars had similar phenologies. Flowering of both cultivars began 40 DAS in 2003 and 42 DAS in 2004. In both years, shoot biomass was not significantly different between the two cultivars until 94 and 96 DAS in 2003 and 2004, respectively. However, the shoot biomass of Chibahandachi was greater than that of Nakateyutaka at 139 and 134 DAS in 2003 and 2004, respectively (Table 1).

The taproot diameter at the basal region increased during secondary thickening period in both cultivars, and became significantly greater in Chibahandachi than in Nakateyutaka at late growth stages: 94 and 139 DAS in 2003 (Fig.1) and 96 and 134 DAS in 2004 (data not shown). Varietal differences of taproot diameter were smaller in the middle and apical parts of the root axis in both years.

The number of the 1st-order lateral roots per unit length of their parental root axis decreased acropetally along the taproot axis in both cultivars, and there were no differences between the cultivars at the corresponding positions on the root axis (Table 2). At early growth stages, varietal differences in the diameter of the 1st-order lateral roots in both cultivars were small at all positions along the taproot axis (Fig. 2). However in the later growth stages, the diameter...
Tajima et al. — Distribution Pattern of Root Nodules in Peanut

of the 1st-order lateral roots at the basal part of the taproot in Chibahandachi than that of Nakateyutaka (Fig. 2).

Only a few nodules formed on the 2nd-order lateral roots, although the 3rd-order lateral roots were not examined because of their scarcity. Root nodules were rarely formed on the taproot, and their maximum number was only 12 and 6 in 2003 and 2004, respectively. Most nodules (about 90%) developed on the 1st-order lateral roots in both cultivars over the growing period (Table 3). In both years, the number of root nodules on the 1st-order lateral roots increased continuously during the growing period, and the number was greater in Nakateyutaka than in Chibahandachi.

Most nodules on the 1st-order lateral roots were formed mostly at the most basal 2 cm of the taproot in both cultivars at 36 DAS. The number of nodules on the 1st-order lateral roots increased acropetally along the taproot axis as the plant grew, and some nodules were formed even on the lateral roots from the middle of the taproot. The increase in the number of such nodules was greater in Nakateyutaka than in Chibahandachi. However, the majority of nodules were formed on the 1st-order lateral roots at the basal region of the taproot (within 8 cm from the base) in both cultivars (Figs. 3 and 4). At the basal 2 cm of the taproot, the number of nodules at 139 DAS in 2003 and 134 DAP in 2004 in Nakateyutaka was 1.5 and 2 times that in Chibahandachi, respectively. The number of root nodules on the lateral roots from the middle of the taproot was greater in Nakateyutaka than in Chibahandachi at later growth stages.

Table 4 shows the distribution of nodule size. Nakateyutaka had a larger number of, but smaller nodules than Chibahandachi throughout the growing period, and the difference in nodule size between the two cultivars became greater with the growth of the plant. In 2003, More than 50% of the nodules were small (<1.0 mm) in both cultivars at 36 DAS.
Chibahandachi had many medium-sized nodules (1.0-2.0 mm) in diameter at 66 DAS and many large nodules (2.0-3.0 mm) at 94-139 DAS. On the other hand, Nakateyutaka had three times as many small nodules (<1.0 mm) as Chibahandachi at 139 DAS. Although the number of medium-sized nodules (1.0-2.0 mm) in Nakateyutaka was similar to that in Chibahandachi, Nakateyutaka had a larger number of small nodules and a smaller number of large nodules than Chibahandachi at 139 DAS. This tendency was similar in 2004 (Table 4).

Fig. 5 shows the relationship between the number of nodules per unit length of the 1st-order lateral roots and the diameter of the 1st-order lateral roots in 2003. Bars indicate standard errors.

Table 3. The number of nodules formed on the taproot and 1st-order lateral roots.

| Year | Cultivar   | DAS  |       |       |       |       |       |
|------|------------|------|-------|-------|-------|-------|-------|
|      |            | 36   | 66    | 94    | 139   | 36    | 65    |
|      |            | tap  | laterals | tap  | laterals | tap  | laterals | tap  | laterals |
| 2003 | Chibahandachi | 4   | 47     | 6   | 134     | 9   | 300     | 12   | 329     |
|      | Nakateyutaka | 3   | 52     | 7   | 206     | 11  | 440     | 11   | 332     |
|      |             | ns   | ns     | ns   | **     | ns   | *      | ns   | ns      |
| 2004 | Chibahandachi | 1   | 24     | 3   | 75      | 4   | 160     | 2    | 202     |
|      | Nakateyutaka | 2   | 35     | 6   | 184     | 5   | 271     | 4    | 282     |
|      |             | ns   | ns     | ns   | **     | ns   | *      | ns   | ns      |

*, **: significantly different between the two cultivars at 0.05, and 0.01 levels, respectively.

Chibahandachi had many medium-sized nodules (1.0-2.0 mm) in diameter at 66 DAS and many large nodules (2.0-3.0 mm) at 94-139 DAS. On the other hand, Nakateyutaka had three times as many small nodules (<1.0 mm) as Chibahandachi at 139 DAS. Although the number of medium-sized nodules (1.0-2.0 mm) in Nakateyutaka was similar to that in Chibahandachi, Nakateyutaka had a larger number of small nodules and a smaller number of large nodules than Chibahandachi at 139 DAS. This tendency was similar in 2004 (Table 4).

Fig. 5 shows the relationship between the number of nodules per unit length of the 1st-order lateral roots and the diameter of the 1st-order lateral roots in 2003. At each growth stage, the number of nodules showed a quadratic correlation with the diameter of the 1st-order lateral roots in both cultivars. Both cultivars had a specific diameter of the 1st-order lateral roots, which is most suitable for nodule formation at each growth stage. The most suitable diameter of the 1st-order lateral roots as the plant grew.

Discussion

Root nodule formation has rarely been studied in relation to the position in a root system. A few investigators reported that most nodules are usually formed on the taproot in many legumes including peanut (Kamata, 1958). However, our observations showed that nodules were rarely formed on the taproot and were mainly formed on the 1st-order lateral roots in both peanut cultivars (Table 3). Root nodules of peanut have been reported to develop mainly at the emergence site of lateral roots after the invasion of microorganisms into the root through cracks on the parental roots, which were caused by the emergence of lateral roots (Boogerd and van Rossum, 1997; Uheda et al., 2001), although nodule formation often occurs at the site of root hair formation in many other
leguminous species. Most nodules of peanut formed on the 1st-order lateral roots in this study, probably because many 2nd-order lateral roots emerged from the 1st-order lateral roots.

The number of nodules on the 1st-order lateral roots was far greater than those on the taproot in this study. Peanut has a deep taproot, but most of the 1st-order lateral roots show a shallow distribution (Ketring and Reid, 1993), and nodules are formed dominantly on the shallow lateral roots. Such a distribution of nodules in the root system possibly occurs because root nodules need a large amount of oxygen for nitrogen fixation and the formation and activity of nodules are often inhibited by low levels of oxygen (Weisz and Sinclair, 1987).

The growth angle to horizon of the 1st-order lateral roots was smaller in Chibahandachi than in Nakateyutaka in our previous study (Tsukamoto et al., 2005). Moreover in the present study, the 1st-order lateral roots of Nakateyutaka collected by the monolith method, in which the root system was cut by the wall of metallic box, were longer than those of Chibahandachi (Table 2). This was probably because the 1st-order lateral roots grow more vertically in Nakateyutaka than in Chibahandachi. Although there are few reports on the formation and development of root nodules in relation to the growth direction of lateral roots, lateral roots with horizontal growth have the advantage of acquiring oxygen necessary for nitrogen fixation as discussed above (Weisz and Sinclair, 1987).

In this study, Chibahandachi had larger nodules
The root nodules in Chibahandachi developed better than those in Nakateyutaka (Table 4). The root nodules in Nakateyutaka had more nodules than Chibahandachi, which suggests that the direction of the 1st-order lateral roots might have no relevance to nodulation.

The site of nodule formation shifts as the plant grows (Bhuvaneswari et al., 1981), indicating that the period of nodule formation at each site is limited. As the plant grows, the position of the 1st-order lateral roots with nodules expanded acropetally along the taproot axis. This tendency was clearer in Nakateyutaka (Figs. 3 and 4), and correlated with the formation of the 2nd-order lateral roots. This might be the reason why Nakateyutaka had smaller, but more root nodules than Chibahandachi.

Chibahandachi had fewer, but larger nodules than Nakateyutaka throughout the growing period. Such a varietal difference became more obvious as the plant grew (Table 4). This result suggests that the nodules in Chibahandachi formed at early growth stages could continue to develop for a long period during plant growth, but the nodules in Nakateyutaka could not. Instead, Nakateyutaka could form many new root nodules, even at late growth stages.

In general, larger nodules have a larger nitrogen-fixing zone than smaller nodules. For example, in soybean, the nitrogen-fixing zone accounts for 25% of the volume in nodules 2 mm in diameter but for 60% of the volume in nodules with 4 mm in diameter (King and Purcell, 2001). Therefore, the larger nodules in peanut must also have much larger nitrogen-fixing zones than the smaller nodules.
fixing activity than smaller nodules. Thus individual
nodules of Chibahandachi should have higher nitrogen-
fixing activity than that of Nakateyutaka, whereas
Nakateyutaka may depend on a larger number of small
nodules to fix nitrogen.

With plant growth, not only the basal diameter of the taproot
but also the diameter of the 1st-order lateral roots became larger (Fig. 1 and 2). The
thickening of the 1st-order lateral roots was more
obvious in Chibahandachi than in Nakateyutaka,
and this varietal difference may have affected nodule
formation and development. The relationship between
number of nodules per unit length of the 1st-order lateral
root and the diameter of the 1st-order lateral roots
(Fig. 5) suggests that each cultivar has a specific
diameter of the 1st-order lateral root optimal for
nodule formation at each growth stage. This suggests
that root system development is specifically related to
nodule formation and development. That is, when the
1st-order lateral roots grew over a certain diameter,
nodule formation was suppressed and nodule
senescence was enhanced. There are only a few
reports regarding nodule formation and senescence
(nodule turnover) (Voisin et al., 2003) and there are
no reports about nodule turnover in relation to root
development. The results of this study indicate that
the diameter of the 1st-order lateral roots is related to
nodule turnover in peanut.

Acknowledgements

We would like to thank Mr. Risei Hasegawa, Mr.
Shigeru Suzuki and Mr. Yoshiharu Iwata (Chiba
Prefectural Agriculture Research Center) for supplying
peanut seeds, and Tokachi Nokyouren for providing
Bradyrhizobium sp. We also thank Mr. Shigeru Hatano,
Mr. Hirofumi Kubota, Ms. Miyuki Shirai, Mr. Hidetoshi
Teshima and Ms. Kaori Sumiyai at Field Production
Science Center, The University of Tokyo, for their help
in growing peanut.

References

Bell, M.J., Wright, G.C. and Peoples, M.B. 1994. The N2-
fixing capacity of peanut cultivars with differing assimilate
partitioning characteristics. Aust. J. Agric. Res. 45 : 1455-1468.
Bhuvaneswari, T.V., Bhagwat, A.A. and Bauer, W.D. 1981.
Transient susceptibility of root cells in four common legumes
to nodulation by rhizobia. Plant Physiol. 68 : 1144-1149.
Boogerd, F.C. and van Rossum, D. 1997. Nodulation of
groundnut by Bradyrhizobium, a simple infection process by
crack entry. FEMS Microbiol. Rev. 21 : 5-27.
Daimon, H., Horii, K., Shimizu, A. and Nakagawa, M. 1999.
Nitrate-induced inhibition of root nodule formation and
nitrogenase activity in peanut (Arachis hypogaea L.). Plant
Prod. Sci. 2 : 81-86.
Daimon, H. and Yoshikawa, M. 2001. Responses of root nodule
formation and nitrogen fixation activity to nitrate in a split
root system in peanut (Arachis hypogaea L.). J. Agron. Crop
Sci. 187 : 89-95.

Hartlarson G, Bliss, F.A., Cigales-Rivelo, M.R., Kipe-Nolt, J.A., Longerri,
L., Manrique, A., Pena-Gabriales, J.J., Pereira, P.A., Sanabria, C.A.
and Tsai, S.M. 1993. Genotypic variation in biological nitrogen
fixation by common bean. Plant Soil 152 : 79-70.
Ikeda, J. 2003. Mathematical description for nodule size
distribution and number weight and diameter of nodules
deduced from a theoretical model of photosynthetic
competition among nodules. Soil Sci. Plant Nutr. 49 : 805-815.
Kamata, E. 1958. Morphological and physiological studies on
nodule formation in leguminous crops. III. The classification
on the basis of the location and the origination in nodule
formation. Jpn. J. Crop Sci. 26 : 255-258*.
Kethering, D.L. and Reid, J.L. 1993. Growth of peanut roots under
field conditions. Agron. J. 85 : 80-85.
Khan, M.K. and Yoshida, T. 1994. Nitrogen fixation in peanut
determined by acetylene reduction method and 15N-isotope
dilution technique. Soil Sci. Plant Nutr. 40 : 283-291.
King, C.A. and Purcell, L.C. 2001. Soybean nodule size and
relationship to nitrogen fixation response to water deficit.
Crop Sci. 41 : 1094-1107.
McDermott, T.R. and Graham, P.H. 1989. Bradyrhizobium
japonicum inoculant mobility nodule occupancy and acetylene
reduction in soybean root system. Applied Environ.
Microbiol. 55 : 2493-2498.
Pazdernek, D.L., Graham, P.H. and Orf, J.H. 1997. Variation
in the pattern of nitrogen accumulation and distribution in
soybean. Crop Sci. 37 : 1482-1486.
Phillips, D.A. 1999. Using Rhizobia in the 21st century. Symbiosis
27 : 83-102.
Reddy, T.Y., Reddy, V.R. and Anbumozhi, V. 2003. Physiological
responses of groundnut (Arachis hypogaea L.) to drought
stress and its amelioration: a critical review. Plant Growth
Regulation 41 : 75-88.
Tamura, Y. 1992. Growth rate analysis of soybean nodules and
particle size distribution. Jpn. J. Soil Sci. Plant Nutr. 63 : 684-689*.
Tsukamoto, Y., Tajima, R., Morita, S and Abe, J. 2005.
The effects of phosphorus deficiency on growth angle of
1st-order lateral roots in peanut (Arachis hypogaea L.). Proceeding
of International Peanut Congress 2005 : 70-71.
Uheda, E., Daimon, H. and Yoshizako, F. 2001. Colonization and
invasion of peanut (Arachis hypogaea L.) roots by guill-marked
Bradyrhizobium sp. Can. J. Bot. 79 : 733-738.
Vikman, P. and Vessey, K. 1995a. Ontogenic changes in root
nodule subpopulations of common bean (Phaseolus vulgaris L.)
1. Nitrogenase activity and respiration during pod-filling.
J. Exp. Bot. 44 : 563-569.
Vikman, P. and Vessey, K. 1995b. Ontogenic changes in root
nodule subpopulations of common bean (Phaseolus vulgaris L.)
3. Nodule formation growth and degradation. J. Exp. Bot. 44 : 579-586.
Voisin, A.S., Salon, C., Jeudy, C. and Wambreum, F.R. 2003.
Symbiotic N2 fixation activity in relation to C economy of
Pisum Sativum L. as a function of plant phenology. J. Exp. Bot.
54 : 2733-2744.
Weisz, P.R. and Sinclair, T.R. 1987. Regulation of soybean
nitrogen fixation in response to rhizosphere oxygen II.
Quantification of nodule gas permeability. Plant Physiol. 84 : 966-970.
Yamagishi, J., Nakamoto, T. and Richner, W. 2003. Stability
and variability of wheat and maize biomass in a small field
managed under two contrasting tillage systems over 3 years.
Field Crops Res. 81 : 95-108.

* In Japanese with English summary.