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Interspecific Differences in Growth and Nitrogen Uptake among *Crotalaria* species*

Hiroyuki DAIMON, Satoshi TAKADA, Masamichi OHE
and Hironori MIMOTO
(*College of Agriculture, University of Osaka Prefecture, Sakai 593, Japan*)
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Abstract : The interspecific differences in germination, root nodule formation, dry matter production and nitrogen uptake were evaluated among *Crotalaria* species for the introduction as new green manure legumes. The germination progressed smoothly in *C. juncea* and *C. spectabilis*, but physical scarification was neccessary in *C. pallida*. In the field experiment, *C. juncea* had a high dry weight and a great nitrogen content at the early growing stage, but a drastic defoliation and a high C-N ratio were found at the late growing stage. *C. pallida* grew slowly at the early stage, but a high nitrogen content and an adequate C-N ratio for decomposition were found at the late growing stage. *C. spectabilis* showed intermediate traits in dry matter production and nitrogen uptake. In the root-box experiment, the distribution patterns of root systems and root nodules varied among the three species. *C. juncea* had greater root growth and nodulation compared with two other species at 35 days after sowing. When the three species are introduced to various cropping systems, these traits should be considered for choice as green manures.
Key words : C-N ratio, *Crotalaria*, Dry matter production, Green manure crop, Nitrogen fixation, Nitrogen uptake.

クロタラリア属植物における生長と窒素吸収の種間差異 : 大門弘幸・高田聡志・大江真道・三本弘乗（大阪府立大学農学部）

要　旨 : 近年我が国において，緑肥作物としての導入が試みられているクロタラリア（*Crotalaria* sp.）の乾物生産ならびに窒素吸収特性について，*C. juncea, C. spectabilis, C. pallida* の 3 種を供試して，種間差異を調査した．圃場試験に先立ち，発芽率を調査したところ，*C. juncea* と *C. spectabilis* は高い発芽率を示したが，*C. pallida* には磨傷処理が必要であった．圃場試験において，播種後 40，80，120 日目には，*C. juncea* が他の 2 種に比べて高い地上部乾物重および全窒素含有量を示したが，播種後 120 日目から 160 日目にかけて著しい落葉が生じ，全窒素含有量の減少が認められた．一方，*C. pallida* は，初期生育が他の 2 種に比べて遅かったが，播種後 120 日目から 160 日目にかけて新葉の抽出が多く，乾物重および全窒素含有量の著しい増大が認められ，この時期の窒素固定量が大きいことが推察された．緑肥の分解速度の律速要因の一つである C-N 率は，播種後 160 日目には，*C. juncea*（33）が最も高く，ついで *C. spectabilis*（27），*C. pallida*（21）の順であった．根箱試験において，生育初期における *C. juncea* の根系発達が他の 2 種に比べて優り，根粒着生も早いことが示された．
キーワード : 乾物生産，クロタラリア，C-N 率，窒素吸収，窒素固定，緑肥作物．

Genus *Crotalaria*, which is originated in India and has now spread to most tropical regions, is an annual or perennial legume. Of many species, *C. juncea* (sunn hemp) is used as a fiber crop, and *C. alata* and *C. burhia* are used as a fodder crop[6,8]. As *Crotalaria* has a high-dry matter production potential and grows vigorously on poor soil with lower nitrogen content and also has nematocidal activity in its roots[6,8,11], it is expected to be introduced as a green manure crop. In fact, *C. juncea* has been introduced to the paddy-upland rotated fields and the ill-drained clayey paddy fields for the improvement of physical conditions and chemical properties of the soils[12,13].

In order to apply the green manures efficiently under the various cropping systems, it is essential to evaluate several ecophysiological traits, such as dry matter production, earliness and nutrient-use efficiency. Especially in leguminous green manure crops, characteristics in relation to nitrogen fixation have to be investigated. Although *Crotalaria* has been known to be antagonistic to nematodes, there are few informations on the properties of nitrogen uptake. The present experiments were designed to clarify the interspecific difference in dry matter production and nitrogen uptake among *Crotalaria* species.

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Materials and Methods

1. Plant materials

Crotalaria juncea, *C. spectabilis* and *C. pallida* were used as plant materials. Seeds of the three species were obtained from Chiba Prefectural Agricultural Experiment Station, Chiba, Japan.

2. Germination test

In order to confirm the germination percentage of the three species, 20 seeds for each species were inoculated on filter paper wetted by distilled water and incubated at 25°C under the illumination of 12 h (20 μmol photons m^{-2} s^{-1}) with fluorescent lamps. Physical treatment was applied to hard seeds of *C. pallida* for promoting the permeability to water. The seeds were scarified in a mortar with a small amount of quartz sand and then a germination test was conducted as described above.

3. Fields experiment

The experiment was carried out in 1992 on the Experiment Farm at University of Osaka prefecture in Sakai. Soil at the site was a silty loam (a gray lowland soil; Haplaquept) with a pH (H_2O) of 5.6, 0.50% T-C, 0.06% T-N and a EC of 23 μS cm^{-1}. One hundred seeds of each species were sown on May 22 on each plot of 70×150 cm in area. The replication was not made for each plot because of limited quantity of seeds obtained. No fertilizer was applied. At 40, 80, 120, and 160 days after sowing, 10 uniform plants for each species were sampled. The sampled plants were separated into leaves, stems, and pods (if present), and oven-dried at 70°C for 2 days. After measurement of dry weight, the samples were ground into fine powder. Total nitrogen and total carbon content were analysed by a Sumigraph Model NC-80 N.C. —Analyzer (Sumitomo Chem. Inc., Osaka, Japan).

4. Observation of root systems and root nodule formation using the root-box-culture technique

Root box (30 cm in depth, 30 cm in width, 1.2 cm in thickness) was filled with mixture of vermiculite and the soil of the experiment field (1 : 2, vol/vol). Superphosphate (500 mg/box) and potassium sulfate (100 mg/box) were added to mixed soil. Seeds of the three species were sown on May 21, 1992. Plants were grown outdoors under natural conditions for 35 days. The profiles of root systems which appeared on the root-box surface were recorded at 20 and 35 days after sowing by tracing on the OHP film (Transparency, Canon Inc., Tokyo, Japan). The experiment was conducted with five replicatons of each species.

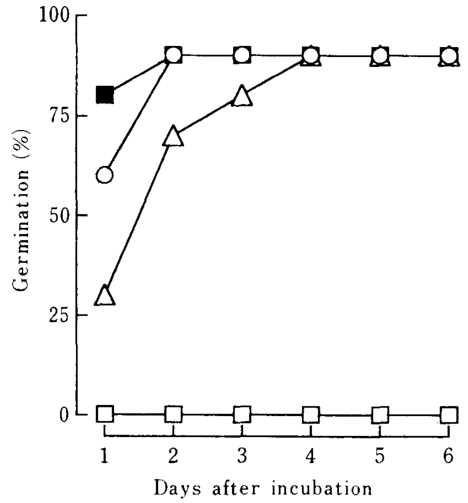

Fig. 1. Changes in germination percentage of *Crotalaria* species.
—○— : *C. juncea*, —△— : *C. spectabilis*,
—□— : *C. pallida*, —■— : *C. pallida*. (scarified)

Results and Discussion

Changes in germination percentage of the three species are shown in Fig. 1. *C. juncea* and *C. spectabilis* exhibited high germination percentage at 2 to 4 days after incubation. On the other hand, *C. pallida* did not show the swelling by water absorption. Physical scarification to seed coat was effective for the increase of permeability to water in *C. pallida*. The scarification with a small amount of quartz sand readily increased germinable seeds to 90%.

Impermeability to water in legume seeds is referred to as hard seed. Several physical and chemical treatments on the hard seeds, such as mechanical scarification, soaking in various solvents and heating at high temperature, have been tried to make them permeable[4,10]. Physical scarification is the most common method, but seeds treated this way are often injured and result in decrease in vigor and viability[3]. In the present experiment, injuries on the hypocotyls were rarely found. In *Crotalaria* species, moreover, it is really difficult to scarify the seed uniformly because of its small size. A rapid, convenient and effective method for making hard seed permeable

Table 1. Changes in plant height and stem diameter of *Crotalaria* species.

	Days after sowing	*C. juncea*	*C. spectabilis*	*C. pallida*	LSD (P=0.05)
Plant height (cm)	40	47.5	16.2	16.1	2.1
	80	166.3	79.1	81.3	8.8
	120	213.3	148.7	148.3	18.7
	160	235.8	150.9	171.4	16.2
Stem diameter* (mm)	40	3.2	2.3	2.2	0.4
	80	9.8	7.9	6.9	1.4
	120	10.2	9.3	11.9	1.8
	160	10.9	10.4	14.1	2.3

*Measured at 5 cm height.

should be developed for a large scale cultivation of *C. pallida*.

In the field experiment, seedlings of *C. juncea*, *C. spectabilis* and *C. pallida* emerged at 3, 7, and 10 days after sowing, respectively. Each species had numerous yellow flowers at anthesis begun on July 13, 22 and 29, in *C. juncea*, *C. spectabilis* and *C. pallida*, respectively. The full-bloom stage was the middle of August (80-90 days after sowing) and pods were produced at the end of September (120-130 days after sowing) in each species.

Changes in plant height and diameter of stem are shown in Table 1. *C. juncea* attained a height of 236 cm at 160 days after sowing. *C. spectabilis* and *C. pallida* showed almost the same patterns on plant height by 120 days after sowing, but the latter was significantly higher at 160 days after sowing. When *Crotalaria* species is grown for green manure, stem diameter may be an important factor for the incorporation into soil. In this experiment, difference in diameter among the three species at 160 days after sowing was found, and the stem diameter of *C. pallida* at 5 cm height was significantly thicker than that for two other species. It was actually difficult for *C. pallida* to be harvested at the ground level by a sickle. The most important factor affecting the value of stem diameter is generally the planting density. If allowed to grow under the condition of high planting density, the value will become lower. Adequate planting density for both high dry matter production and easier incorporation should be examined.

Changes in top dry weight of the three species are shown in Fig. 2. At each sampling date, the total dry weight of *C. juncea* was higher than that for two other species, and the differences were remarkable at 80 and 120 days after sowing. The rise in the dry weight from 80 to 160 days after sowing was mostly caused by an increase in stems in each species. Defoliation was found from 120 to 160 days after sowing in *C. juncea* and *C. spectabilis* and it was drastic in *C. juncea*. In *C. pallida*, however, emergence of new leaves was found continuously and foliage was dark green in color at 160 days after sowing. In this experiment, the quantitative evaluation of defoliated leaves was not conducted because it was difficult to estimate accurately in the field conditions.

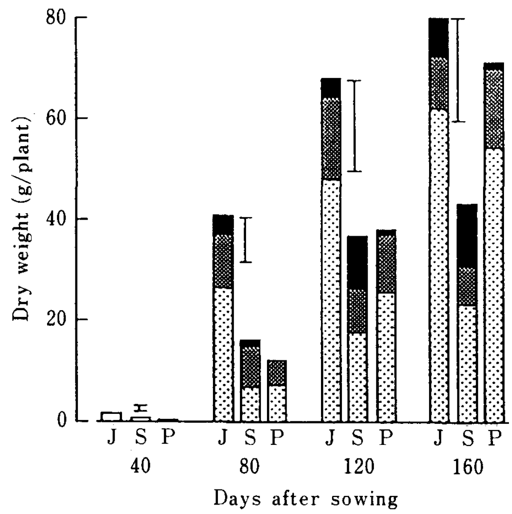

Fig. 2. Changes in top dry weight of *Crotalaria* species. Vertical bar indicates LSD of dry weight of whole plant on each sampling date (P=0.05).
J : *C. juncea*, S : *C. spectabilis*, P : *C. pallida*,
▨ : Stem, ▩ : Leaf, ■ : Pod.

Changes in top nitrogen content of the three species are shown in Fig. 3. The amount of nitrogen in the whole plant of *C. juncea* was significantly higher than that of two other species at 40, 80 and 120 days after sowing. At

160 days after sowing, it was slightly decreased because of a reduction in leaf nitrogen due to the defoliation. In *C. pallida*, on the other hand, nitrogen content increased remarkably from 120 to 160 days after sowing, both in the whole plant and in each part.

Changes in C-N ratios of the whole plants in the three species are shown in Table 2. The C-N ratios gradually increased throughout the growing period in each species. At 160 days after sowing, the ratio of *C. juncea* was 32.5, followed by *C. spectabilis* of 27.3, and *C. pallida* of 20.5. Especially in *C. pallida*, the lowest value of 20.5 was noteworthy. The C-N ratio is usually used for an indicator of the decomposing rate of green manures, and a value greater than 25 might be critical for rapid decomposition[1,2,9]. We reported previously that high C-N ratio in *C. juncea* was one of the causes for lower nitrogen uptake of the succeeding wheat on the *C. juncea*-wheat rotation plot compared with the peanut-wheat plot[14]. A lower C-N ratio of less than 25 during the later growing stage in *C. pallida* may be a desirable trait for green manure.

Since no nitrogen fertilizer was applied to the fields throughout the growing period and soil of the field contained a small amount of nitrogen (0.06% T-N), the nitrogen absorbed by each species was derived mainly from nitrogen fixed by *Crotalaria*-rhizobia symbiosis. In other words, the differences in the amount of nitrogen accumulated among the three species in the present study might be based on the ability of nitrogen fixation. Further studies for the quantitative estimation of nitrogen fixed in the three species are now in progress.

In the root-box experiment, the distribution patterns of root systems varied among the three species. The moderate profiles of root systems at 20 and 35 days after sowing are shown in Fig. 4. The root mass was apparently greater in *C. juncea* compared with *C. spectabilis* and *C. pallida*. At 20 days after sowing, several root nodules were found on the primary and lateral roots in *C. juncea* and only on the primary root in *C. spectabilis* and *C. pallida*. The number of root nodules in each species remarkably increased during 15 days after the first sampling date, and then almost all nodules had a characteristic pink color owing to the presence of leghaemoglobin, which is a constant and prominent feature in the central tissue of all nitrogen fixing leguminous nodules.

This result showed that effective root nodule bacteria to *Crotalaria* existed in the experimental field. Soybean (*Glycine max*) and peanut (*Arachis hypogaea*) had been cultivated in the field but *Crotalaria* species had never been grown prior to this experiment. Affinitive relationships between plant genotypes and rhizobial species in the various combinations are well known. The cross-inoculation grouping among the three plant species remains unknown, though Rhizobia from *Glycine*, *Arachis* and *Crotalaria* were grouped as *Bradyrhizobium* spp[5,7]. Further work is necessary for

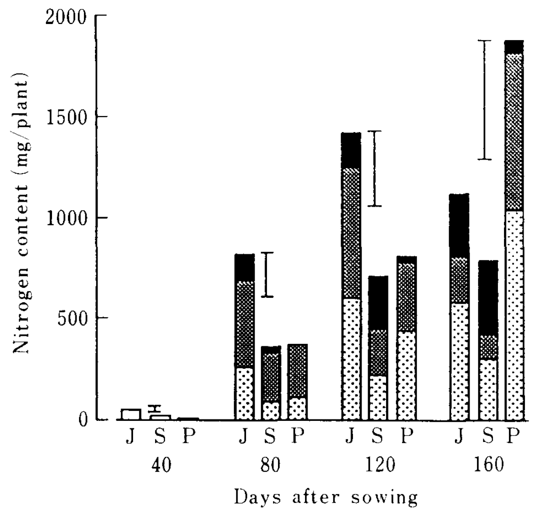

Fig. 3. Changes in top nitrogen content of *Crotalaria* species. Vertical bar indicates LSD of nitrogen content of whole plant on each smpling date (P=0.05).
J : *C. juncea*, S : *C. spectabilis*, P : *C. pallida*,
▨ : Stem, ▩ : Leaf, ■ : Pod.

Table 2. Changes in C-N rations of the whole plants of *Crotalaria* species.

Days after sowing	*C. juncea*	*C. spectabilis*	*C. pallida*	LSD (P=0.05)
40	12.2	11.3	11.0	1.1
80	21.6	18.7	14.0	1.3
120	23.5	22.9	22.3	2.5
160	32.5	27.3	20.5	4.9

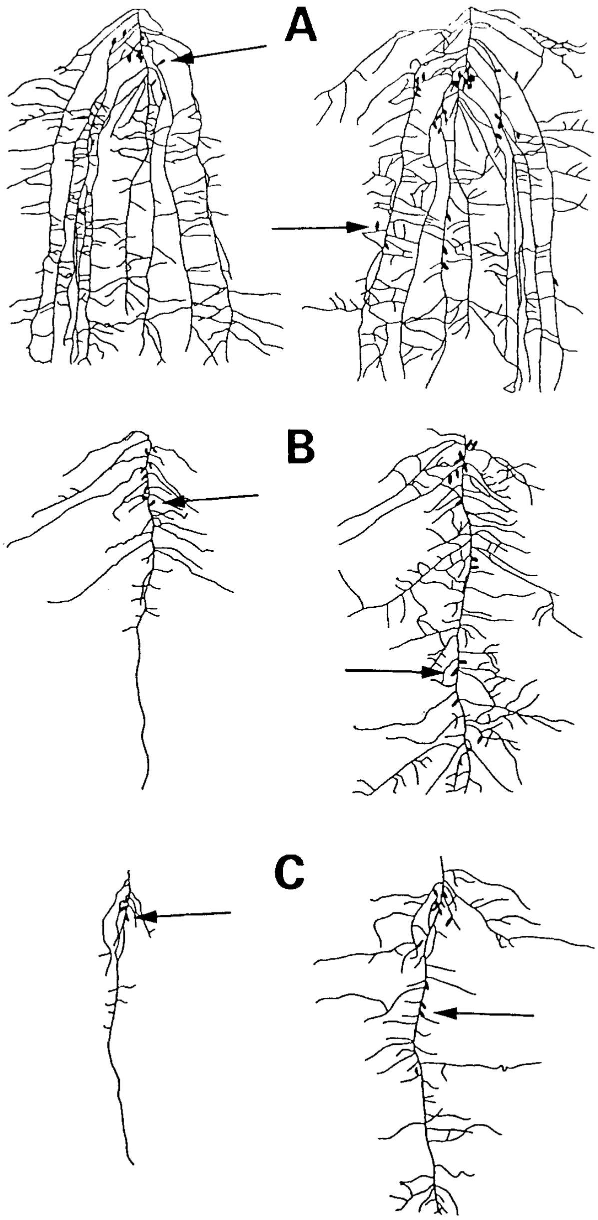

Fig. 4. The profiles of root systems in *C. juncea* (A), *C. spectabilis* (B) and *C. pallida* (C) at 20 (left) and 35 (right) days after sowing. Arrows indicate root nodules in each species.

the host-specificity of rhizobia distributed in the present experimental fields.

The root growth by depth and lateral spread is an important factor for improvement of soil physical properties. *C. juncea* cultivated on ill-drained clayey paddy fields in the Hokuriku district had a deeper and prolific lateral root branching system[12]. In the present study, *C. juncea* also had greater root growth and nodulation compared with two other species in the early growth stages as shown in Fig. 4. Throughout the whole growing period, various characteristics of root systems such as root mass, lateral branching and nodulation must be evaluated to introduce these legumes as green manure.

In summary, *C. juncea* had a high dry weight and a great nitrogen content at the early growing stage because of its high ability of nitrogen fixation, but a drastic defoliation and a high C-N ratio at the late growing stage should be noted. On the other hand, *C. pallida* grew slowly at the early stage probably due to a slower root branching and nodulation, but had a high nitrogen content and an adequate C-N ratio for decomposition at the late stage. *C. spectabilis* showed intermediate traits between these two species in dry matter production and nitrogen content.

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