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Nitrogen fixation, and growth characteristics of Three Legume cover crops in no-tillage paddy field

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ABSTRACT : A field experiment was conducted to investigate the performance of three legume species in a zero-tillage, non-fertilized rice field in a temperate zone. Before the experiment for 5 years, from 1995 to 1999, plant growth patterns of three legume species grown as over-wintering (October-May) cover crops on a paddy field were maintained to study N balance and N_2 fixation. Decrease in plant density accelerated from after winter to flowering from 1,090, 320, and 5 to 732, 232, and 6 plants m^{-2} in Chinese milk vetch (CMV), white clover (WC), and hairy vetch (HV), respectively. Total dry weights of plants above-ground level were 0.05, 0.11, and 2.43 g $plant^{-1}$ in WC, CMV and HV respectively but steeply increased at ripening up to 0.77, 2.33, and 26 g $plant^{-1}$. The root dry weight of HV and CMV rapidly increased while in WC, root dry weight increased slightly towards flowering. The highest nodule numbers were recorded in CMV to April thereafter WC produced the highest. Nodule size was distributed within 7 mm in CMV but it was larger in HV varying from 1 to 10 mm. Shoot N (g m^{-2}) greatly increased from over-wintering to flowering in CMV, HV and WC and it ranged from 1.66, 0.5 and 1.92 to 12.6, 3.1 and 13.02 g m^{-2}, respectively. After wintering, the initial shoot N content (%) was more in CMV. Root N content (%) was constant or slightly decreased in HV and WC. Soil total N in the control plot (clean fallow) was the highest on Mar. 2 then decreased rapidly to flowering. Soil N content was constant in HV plots whereas it was low in WC plots for the entire growth period except just after winter. Maximum nitrogenase activities were 9, 37.8, and 13ì mol C_2H_4 $plant^{-1}$ $hour^{-1}$ in CMV, HV, and WC, respectively. Nitrogenase activity showed a direct correlation with nodule number, size and fresh weight. As a cover crop preceding a rice crop, CMV is more suited to colder regions due to its earlier ripening characteristics. Hairy vetch and WC are recommended for regions with a mild winter and a long summer owing to their late ripening and great N fixation activity.

Keywords : Chinese milk vetch, nitrogenase activity, narrow-leaf vetch, nitrogen content, N_2 fixation, no-tillage, rice, white clover

Legumes can be grown in rotation with one or more rice crops per year. Legumes, in particular legume green manure, have been traditional nitrogen sources in rice-based cropping systems in Asia. (Watanabe and Liu, 1992). However, the use of legume green manure is declining globally (Becker *et al.,* 1995). Long-term maintenance of soil N fertility in paddy fields is attributable to both N application and natural N supply. The natural supply of N in long-term N-deficient paddy plots in Japan is estimated to range from 49 to 91 kg ha^{-1}, or an average of 70 kg ha^{-1}, per year (Matsuo and Takahashi, 1977). Among the processes by which N is naturally supplied, biological N_2 fixation is a crucial one. In N-balance studies in Japan, annual N_2 fixation in a no added-N fertilizer paddy plot was about 45 and 56 kg ha^{-1} in Kagawa and Shiga, respectively (Hirano, 1958), while in paddy plots with heavy doses of ^{15}N-fertilizer in Saga, NFA (nitrogen fixation activity) was 60-70 kg N ha^{-1} (Matsuguchi and Shimomura 1977). With the use of C_2H_2 reduction method, the NFA rate (ARA. 3 : 1) in a paddy field was 63 kg N ha^{-1} during the dry season (Yoshida and Ancajas 1973). A wide range of NFA rates (ARA. 3 : 1) (0.5 to 54 kg N ha^{-1} $crop^{-1}$), however, was found in paddy fields in Thailand (Matsuguchi *et al.,* 1975). However, Vance (1998) postulated that in agricultural set-ups, perhaps 80% of this biologically fixed N_2 comes from symbioses involving leguminous plants and species of *Rhizobium, Bradyrhizobium, Sinorhizobium, Azorhizobium, Mesorhizobium and Allorhizobium.*

Even though soil acidification by legumes could be a problem (McLay *et al.,* 1997; Helyer and Porter, 1989; Porter *et al.,* 1995; Tang, 1997), the inclusion of leguminous crops into rice-based cropping patterns may contribute towards improving the prospects of their long-term sustainability, primarily because legumes are able to fix atmospheric N and also they are diverse, capable of playing a role in varied agricultural systems (Chalk, 1998; Graham and Vance, 2000; Haynes *et al.,* 1993; Kinzig and Socolow, 1994). The above ground residues of legume crops represent a major source of nutrients for the following rice crops (Becker *et al.,* 1994; Chen, 1988; Cho *et al.,* 2001; Garrity and Flinn, 1988; Jeong *et al.,* 1996; Ockerby *et al.,* 1999; Schultz *et al.,* 1999; Shimizu and Okino, 1994; Wortman *et*

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al., 2000; Yasue, 1991). Additionally, legumes in rotation with rice can increase soil organic matter and reduce pest and insect levels (Hidaka, 1997; Schulz *et al.*, 1999). They are usually grown as dry season crops immediately following single or double lowland rice crops. In sub-tropical and temperate areas, especially, China, Japan and Korea, the season of rice-growing is restricted by low winter temperatures (Chen, 1973; Schulz *et al.*, 1999; Yasue, 1991). Chinese milk vetch (*Astragalus sinicus* L.), hairy vetch (*Vicia villosa* Roth.), and white clover (*Trifolium repens* L.) are leguminous plants that grow commonly in drained paddy fields and levees in Korea, Japan and China with Chinese milk vetch (CMV) in particular. The acreage in Japan was nearly 220,000 and 20,000 ha in 1960 and 1989, respectively (Yasue, 1991). Utilization of CMV as a green manure in paddy fields has been drastically decreased with an increasing supply of nitrogen fertilizers. Since the 1970s when supply of fossil energy resources became critical, the importance of nitrogen fixing plants in the natural ecosystem has been recognized. There are many reports on soil fertility by the natural supply of N. Numerous investigations have been carried out to assess the productivity, N_2 fixation and residual effects of mainly tropical species grown in rotation with rice and comprehensive reviews in this area have been prepared by Buresh and De Datta (1991), Giller and Wilson (1991) and Peoples and Herridge (1990). Commonly grown green manure and food crops include *Sesbania* spp., ricebean (*Vigna* spp.), lentil (*Lens culinaris*), soybean (*Glycine max*) and *Phaseolus* spp. A large number of legume genera such as *Aeschynomene* and *Desmodium* are also grown for forage purposes (Schulz *et al.*, 1999). Major species grown for green manure in Asia are *Sesbania* spp., *Crotalaria juncea, Aeschynomene* spp. and *Astralagus sinicus* (Yadvinder *et al.*, 1991). Jo *et al.*, (1980) and Sasakawa (1987) reported the ARA of CMV both in the pot and paddy fields. However, there is little information with regard to the performance of temperate legume species in rice-based cropping systems and on the nitrogen fixing activity and growth patterns of CMV, Hairy vetch (HV), and White clover (WC) grown in no-tillage rice-based legume-rice cropping systems.

In this research, I evaluated plant growth, ARA and N balance (soil and plant) in CMV, HV, and WC in a no-till, legume-rice cropping system.

MATERIALS AND METHODS

Site characterization

The data in this paper were obtained from experiments conducted in 2000 at the Ehime University Experimental farm, Hojo, Japan. The site (33°57'N, 132°47'E, 15 m asl) is located in a temperate zone with hot humid summers and cold dry winters. For the period 1995-1999, three legume species, CMV, HV, and WC were grown during the winter season (October-May for CMV and HV and October-June for WC). The soil at the experimental site was a sandy loam (sand, 58.5%; silt, 28.0%; clay, 13.5%) with a bulk density of 1.10 g cm^{-3}, taken from a 0.2-m depth, organic carbon content as 20 g kg^{-1} and pH 5.3 (1 : 5 soil : water). Initial total N, available P_2O_5 and exchangeable K, Ca, and Mg in this layer were 0.1%, 102 mg kg^{-1} and 0.3, 2.5 and 0.7 cmol (+)/kg in 1999. Temperature and rainfall data are shown in Fig. 1.

Experimental design, treatments and crop culture

Chinese milk vetch, Hairy vetch, and White clover were established under unfertilized and zero tillage condition in a rice field. Before the commencement of the experiment, these green manures were rotated with rice for 5 years. Seeds of CMV, HV, and WC were broadcast at the recommended rates of 30, 80 and 20 kg/ha, respectively on the paddy field just one week before harvest in October, 1999. The experiment was a completely randomized block design with 3 legume treatments and 5 replications per treatment. The control plots were kept clean fallow by periodic weeding between Feb. 25 to May 16 for CMV and HV and until June 6 for WC. Plots were 3 by 3 m in size.

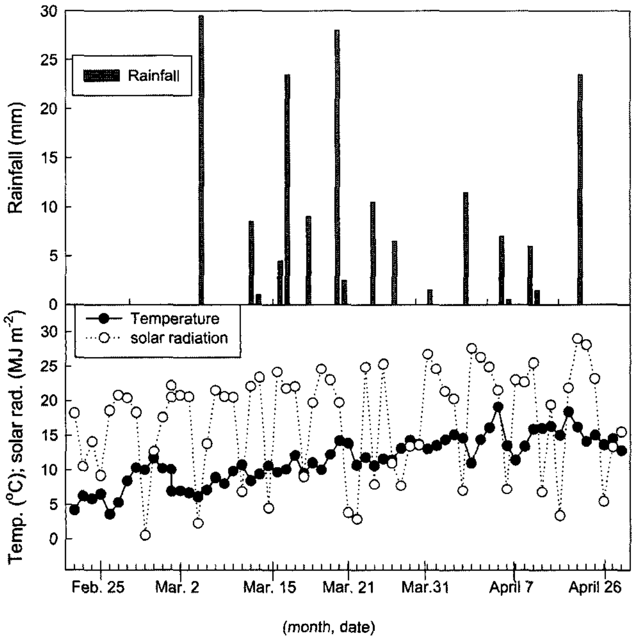

Fig. 1. Rainfall (mm^{-1}), mean daily temperature (°C), and solar radiation (MJ $m^{-2}d^{-1}$) during the wetch cropping season in 2000 in Hojo, Japan.

Measurement of plant, soil characteristics and nitrogenase activity

Plants were harvested around 12-2 p.m. on sunny days from over-wintering (Feb. 22) to ripening. Nitrogen fixing activity of nodulated roots and nodules was determined within 5 min. after harvest. The shoot portion was cut off at the base of plants and soil attached on the roots was removed carefully by hand.

Nitrogen fixing activity (NA) was measured with acetylene reduction method as described by Hardy *et al.*, (1973) for 5 replications per treatment. Nodulated roots were placed in a 1-L polypropylene mason jars and sealed with rubber stoppers. One hundred ml of air was removed using a 50-mL syringe and replaced with 100-mL of C_2H_2. The mason jars were placed in holes from which plants had been excavated, covered with soil, and incubated at soil temperature for one hour. At the end of the incubation period, jars were removed from the soil and after proper mixing 1-mL samples were withdrawn for the analysis of ethylene concentration. Samples were analyzed within 4 h by injecting 1-mL of gas into a gas chromatograph (SHIMADZU, Japan) with GC condition, temperature, carrier gas speed and column type, being similar to those described by Hosoda *et al.*, (1978). A standard of C_2H_4 gas was used for the GC analysis.

Total dry matter and C/N ratio of plant were determined at the time of harvest by removing 10 plants from 0.5 by 0.5 m in CMV and WC and 10 plants from non-considered area in HV plot. Plant height and root length of these plants were measured. Plant samples were separated into roots and shoot parts then dried at 75°C for two days. All dried plant samples were ground before determining the C/N ratio.

After the sampling of the 10 legume plants in each plot, soil samples were collected from 0 to 20 cm depth. After removing large visible pieces of plant material, the soil was dried at indoor and ground to <2 mm for determination of total N and C by the combustion method using a Sumigraph C/N analyzer (Sumigraph NC-90A). All results were reported on oven-dry weight basis.

Nodule size and weight and root length were calculated from the 10 plants. Plant and weed densities were determined from 0.5 by 0.5 m^2 plots as that of weed density measurement; leaf blade damage (%) by insect pests was determined from the percentage of number of damaged leaf blades to the total leaf number.

Nitrogen fixing activity of nodules

Nodules were separated from the roots and classified into eight groups according to their size (diameter). The groups were classified as 0-1, 1-2, 2-3, 3-4, 4-5, 5-6, 6-7 and >7 mm for the 1st, 2nd, 3rd, 4th, 5th, 6th, 7th and 8th group, respectively. Nodules were incubated for 1 h at 20°C at dark condition for the determination of ARA and measuring methods were similar to 2.3 above mentioned.

RESULTS

Plant density, dry weight and N content of vetch

Population density changed from 1,090, 320 and 5 after winter to 732, 232, and 6 plants m^{-2} at flowering in CMV, WC and HV respectively (Fig. 2).

Total above-ground dry weight after winter was 0.05,

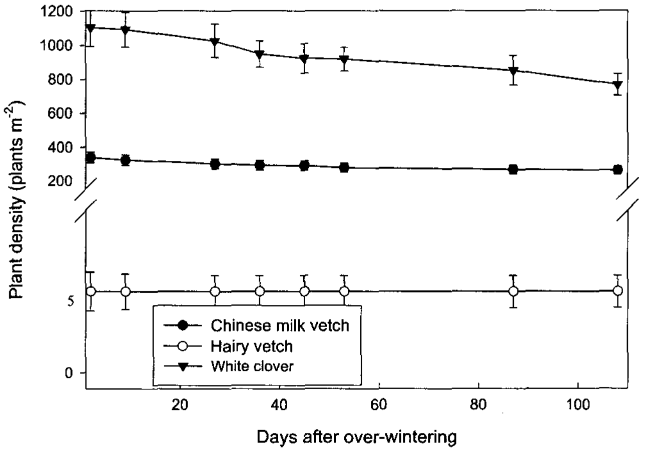

Fig. 2. Change in plant density of Chinese milk vetch (CMV), Hairy vetch (HV) and White clover (WC) in no-tillage paddy field.

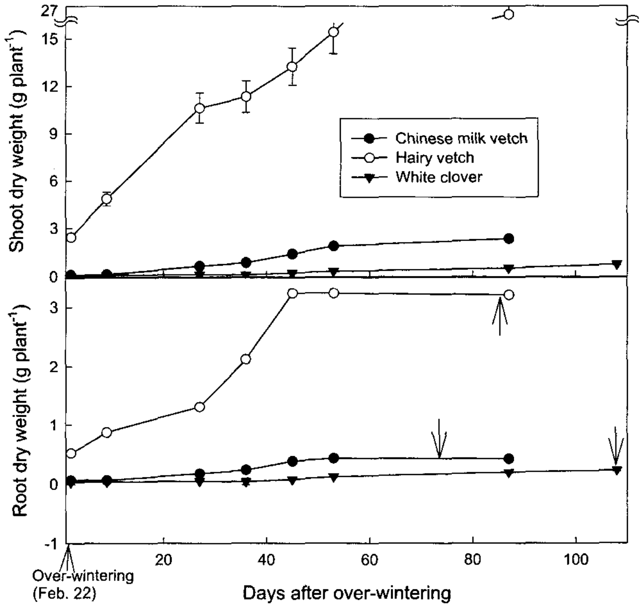

Fig. 3. Change in plant weight of Chinese milk vetch (CMV), hatch (HV), and white clover (WC) in no-tillage paddy field. Arrows indicate flowering time of each legumes.

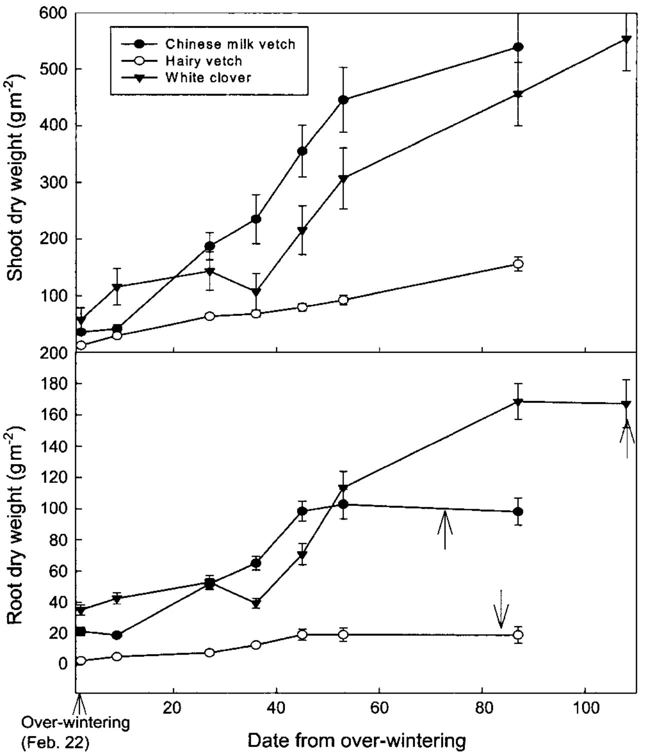

Fig. 4. Change in shoot and root dry weight of Chinese milk vetch (CMV), Harry vetch (HV), and White clover (WC) grown in no-tillage Paddy field. Arrows indicate flowering time of each legumes.

0.11, and 2.43 g plant^{-1} in WC, CMV and HV, respectively, and at flowering, it was 0.77, 2.33, and 26 g plant^{-1}, respectively(Fig. 3). Dry weight per plant increased markedly in HV followed by CMV and WC. Change in root dry weight followed 0.067 to 0.42 g plant^{-1} for CMV, 0.03 to 0.23 g plant^{-1} for WC but 0.52 to 3.2 g plant^{-1} for HV and it was similar to plant dry weight trend(Fig. 3).

There was a slight increase in dry weight (g m^{-2}) for HV, while CMV and WC above ground total dry weight increased rapidly from 35.6 and 57.3 up to 539 and 554 g m^{-2}, respectively, towards ripening(Fig. 4). Root dry weight of HV and CMV increased rapidly, on the contrary, it was constant in WC or slightly increased towards flowering (Fig. 4).

Changes of nodule size and numbers

Nodule size of vetches was larger in HV followed by CMV throughout the growing period. The smallest nodule size was observed in WC(Table 1). Above 6 mm nodule was mostly observed after March in HV and this was the biggest of all legumes. The higher number of nodules per plant was observed in CMV after wintering to April, thereafter the highest number of nodules was recorded in WC. In CMV, nodule size was mostly smaller than 7 mm, but in HV, it was broadly distributed from 1 to 10 mm.

Table 1. Changes in nodule size (mm) and numbers of Chinese milk vetch (CMV), hairy vetch (HV), and white clover (WC) grown in no-till rice field.

Nodule Size(mm)		0-1	1-2	2-3	3-4	4-5	5-6	6-7	>7 mm	l.s.d. (P=0.05)
Treatments		number/plant								
Feb. 21	CMV	4.6	2.7	2.8	2.0	0.0	0.0	0.0	0.0	2.3
	HV	4.4	1	0.0	0.0	0.0	0.0	0.0	0.0	1.7
	WC	6.6	3.8	1.5	0.0	0.0	0.0	0.0	0.0	3.9
Feb. 29	CMV	3.5	2.8	1.3	0.7	0.0	0.0	0.0	0.0	2.6
	HV	2.5	3.0	1.0	3.0	1.7	0.0	0.0	0.0	1.3
	WC	4.8	0.2	0.0	0.0	0.0	0.0	0.0	0.0	2.1
Mar. 18	CMV	4.0	3.7	3.3	1.5	0.0	0.0	0.0	0.0	2.4
	HV	7.3	2.5	3.0	5.3	0.0	3.0	1.0	0.0	3.4
	WC	4.3	3.7	0.0	0.0	0.0	0.0	0.0	0.0	2.7
Mar. 27	CMV	9.0	5.3	2.7	0.0	0.0	0.0	0.0	0.0	3.8
	HV	2.5	1.3	4.0	2.5	5.0	1.5	2.0	0.0	1.8
	WC	7.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.6
April 5	CMV	6.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.3
	HV	4.7	2.0	1.0	0.0	0.0	1.0	0.0	0.3	2.2
	WC	5.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.1
April 13	CMV	11.1	2.8	1.0	0.2	0.0	0.1	0.1	0.0	3.5
	HV	6.0	1.3	0.8	0.2	0.7	0.1	0.1	0.3	2.4
	WC	5.0	1.0	0.1	0.0	0.0	0.0	0.0	0.0	2.2
May 1	CMV	0.0	3.0	2.0	1.0	0.0	0.0	0.0	0.0	1.2
	HV	0.0	2.0	1.0	1.0	0.0	0.0	0.0	1.0	1.1
	WC	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4
May 16	CMV	0.0	16.3	2.7	0.0	0.0	0.0	0.0	0.0	5.2
	HV	0.0	2.0	2.0	1.0	0.0	0.0	0.0	0.0	1.3
	WC	10.5	10.2	0.0	0.0	0.0	0.0	0.0	0.0	3.9

Changes in N content of plant and soil

Above ground total shoot N (g m^{-2}) significantly increased from over-wintering to flowering in CMV and WC and ranged from 1.66 and 1.92 to 12.6 and 13.02 g m^{-2}, respectively (Fig. 5). However, N accumulation in HV was very slow and it ranged from 0.5 to 3.1 g m^{-2} due to the lower plant density during the entire growth period. Root N also similarly inclined to the shoot N, and changed from over-wintering to flowering from 1.02, 0.11, and 0.92 to 3.28, 0.68, and 4.09 in CMV, HV, and WC, respectively, but it ranked about 30% of shoot part N.

After over-wintering, the initial shoot N content (%) in CMV was more than 4.7%. A slow decline in shoot N for all treatments was observed until flowering (Fig. 5). However, there was a rapid decline in shoot N of HV, a marked decline occurred a week after over-wintering from 4.2 to 3.3% then becoming stable or declining slowly until flowering. Root N content (%) was maintained or a little declined until flowering in HV and WC, however there was a temporary increase in root N towards the end of March; about 3-4 weeks before flowering in CMV.

Soil total N content (%) ranged from 0.11 to 0.17 after wintering. Nitrogen in Check plot was greatest with 0.17% on early March then rapidly decreased to 0.12% (Fig. 5). Of all vetch plots, N content was almost 0.13-0.10% and maintained in CMV plot but it was the lowest in WC in entire growth period except just after wintering.

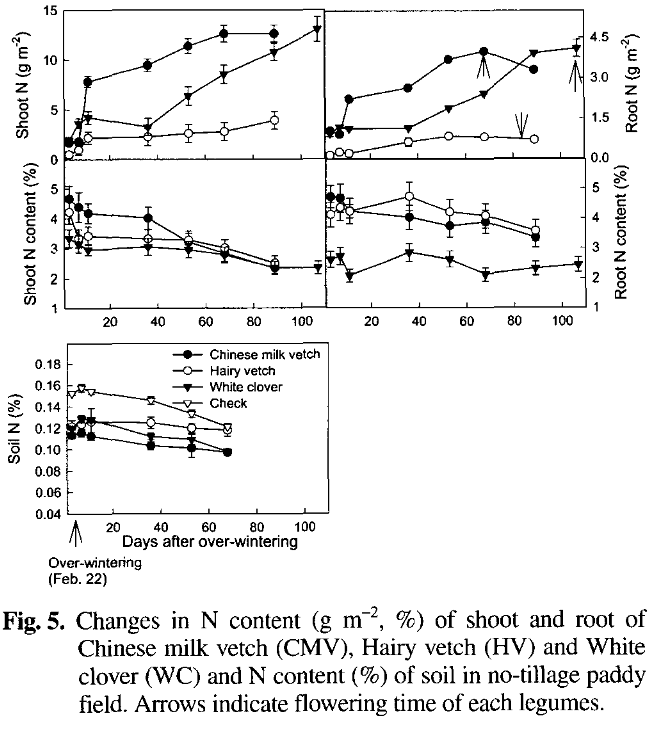

Fig. 5. Changes in N content (g m^{-2}, %) of shoot and root of Chinese milk vetch (CMV), Hairy vetch (HV) and White clover (WC) and N content (%) of soil in no-tillage paddy field. Arrows indicate flowering time of each legumes.

Weekly changes of Acetylene Reduction Activity (ARA) and nodule size characteristics

At the time of just over-wintering (Feb. late), ARA was observed around 0.3 μ mol C_2H_4 $plant^{-1}$ $hour^{-1}$ in all the treatments then it rapidly increased maximally up to 9, 37.8, and 13 μ mol C_2H_4 $plant^{-1}$ $hour^{-1}$ in CMV, HV, and WC, respectively (Fig. 6). Exceptionally, until the flowering, ARA in HV was lower on March 18 and April 5 than the other sampling dates (23, 47 and 75 days after wintering of CMV, HV and WC, respectively) and it was mostly related to the higher soil moisture content after rainfall. However, it increased most slowly in WC, especially, steeply increased after May. In CMV and HV, ARA rapidly increased and was greatest on Mar. 27 then declined rapidly up to flowering time. Soil moisture content (%) was more on Mar. 18 then decreased and was in an inconsistent soil moisture range from 22.6 to 32 % until May 16 (Fig. 6) was observed.

Acetylene reduction activity was mostly correlated with the nodule size (Fig. 7). Nodule size was at maximum in HV followed by CMV and ranged between 1-8 mm, but mostly distributed as 1-3 mm nodule size in WC.

DISCUSSION

Plant density was considerably higher in WC as compared

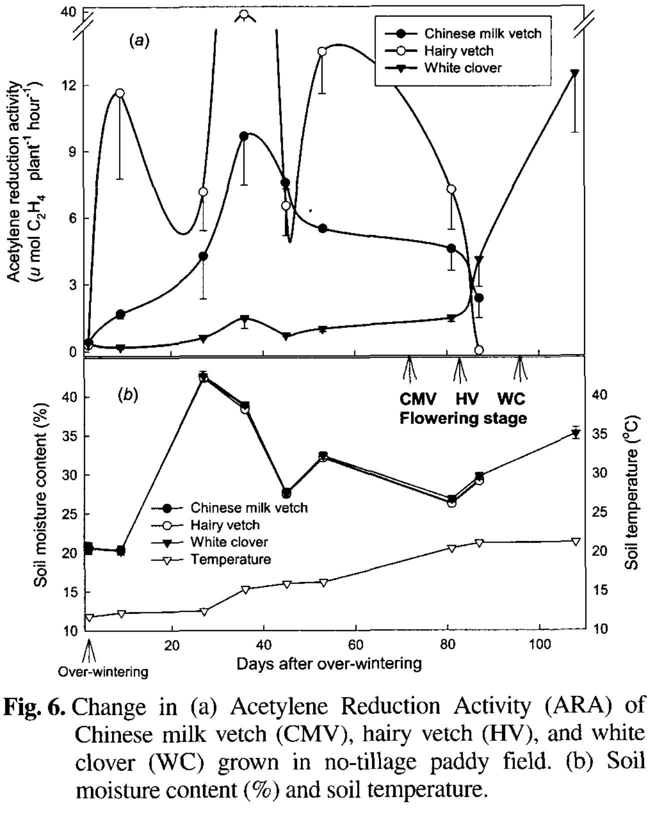

Fig. 6. Change in (a) Acetylene Reduction Activity (ARA) of Chinese milk vetch (CMV), hairy vetch (HV), and white clover (WC) grown in no-tillage paddy field. (b) Soil moisture content (%) and soil temperature.

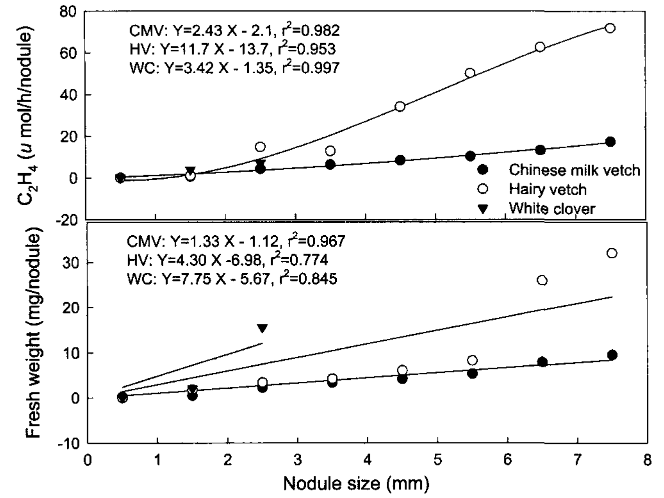

Fig. 7. Influence of nudule sixe on acetylene reduction activity (ARA) and fresh weight of Chinese milk vetch (CMV), hairy vetch (HV), and white clover (WC) grown in no-tillage paddy field.

to the vetches, most likely reflecting lower height and biomass per plant. The reduction in plant density in HV due to bird invasion was compensated by the greater biomass yield per plant towards late spring (Fig. 2). In another study, plant density in zero-tilled plot was lower than in the tilled CMV plots probably because seed germination was hampered by soil compaction and an accumulation of un-degraded rice straw (Cho *et al.*, 2001). Higher plant mortality and leaf defoliation expressed as low plant density could be attributed to the heavier rainfall witnessed over the duration of the experiment (Fig. 1) (Gibberd and Cocks, 1997). The greater biomass in WC compared with CMV and HV was clearly associated with higher plant density (Fig. 4).

Dry weight per plant increased rapidly with a decrease in plant density. Flowering was early in CMV (42-d AW) followed by HV (80-d AW) and was latest in WC (>100-d AW). Legume flowering time is an important consideration under zero tillage legume-rice cropping system because the legume should not impede the proper establishment of transplanted rice seedlings. The rice crop may also fail to utilize the nutrients derived from legume residues if there is insufficient time between legume senescence and rice cropping. Thus, time of transplanting and rice cultivar will determine the legume to be used in such a cropping system.

In WC, nodule size was the smallest among the three species for the whole duration of growth, but both biomass yield and shoot N were intermediate up to flowering stage for CMV as well as HV. Nodule size was higher in WC after flowering time than in both the legumes as a slight delay in flowering pattern and a higher plant density per unit area.

Large ARA responses to total plant density in WC with large increases in nodule number progressed to flowering, and the relatively higher soil temperatures at flowering also contributed to ARA (Fig. 6). There was a strong positive correlation between ARA and nodule size. Moisture content of nodule was 71-73% for the duration of growth (Data not shown). Nodule shape and size varied even within the same cultivar possibly due to changes in soil and environmental conditions, e.g. rounded for HV and WC but circular/conical for CMV.

As indicated by Haynes *et al.*, (1993), most of the plant N absorption occurred after over-wintering in the form of N_2 fixation or root absorption from the soil. The early maturing characteristics of CMV explain its use for green manure in China, Japan, and Korea as opposed to the late maturing HV and WC. Utilization of total biomass and nutrients of HV and WC is not suit in a no-till direct-sown legume-rice rotation cropping, both due to their late maturing nature and the fact that when not fully ripened, they could release organic acids or other inhibitory (i.e., allelopathic) compounds (Chou *et al.*, 1977; Janovicek *et al.*, 1997). Hower, well management of irrigation timing and water depth could overcome this problem (Cho *et al.*, 2003). Chinese milk vetch on the other hand ripens early and in late May, making an ideal candidate for use in paddy cultivation (Cho *et al.*, 1999; Yasue, 1991). There was a great increase in root N towards flowering in WC treatments and it was mostly correlated to nodule number and size. There was an extensive lodging of CMV at flowering time and the heavy rain exacerbated further damage on plant shoots and roots causing leaf defoliation and plant death. Hairy vetch plants lodged in early April taking up an area of 0.3 m^2 per plant. Due to the high density and erect growth pattern, WC could be ideal for inhibiting weed germination and establishment. Root N (%) was slightly changed with ripening due to an increase in the root nodule N for all legumes.

Soil total-N (%) was steeply decreased from 0.15 to 0.13% between early and late March (38 to 55 days after wintering; Fig. 5) in the bare control plot. However, there was no significant change in soil N in the control plot mulched with rice straw (from 0.16 to 0.15, data not shown) due to stable soil temperature and moisture and the entry of N from the decaying rice straw into the soil N pool. Rapidly reduced soil N content between over-wintering and 70-d after in bared control plot was caused by leaching, nitrification, de-nitrification, and volatilization with the fluctuation in day and night temperatures and alternate dry and moist soil conditions after the start of spring rainfall (Bacon and Osborne, 1987). After wintering, soil N content was lower in legume-cultivated plots than in the bare control plot from the legume uptake. However, soil N content remained almost constant in HV plots but was lowest in WC plots probably because of the higher plant density in WC plots.

The positive correlation between nodule size and nodule fresh weight was because of the increase in rhizobium populations and a corresponding increase in nodule size.

CONCLUSIONS

Chinese milk vetch and WC can provide more than 12 and 13 g N m^{-2}, respectively, while HV could provide only 3.1 g N m^{-2} due to low plant densities at maturity. The greater nodule numbers in CMV and WC contributed to the increased N_2 fixation. Acetylene reduction activity per plant was greater in HV but it did not effectively contribute to the improvement of total N content per area due to the lower plant density. Chinese milk vetch could be useful in areas with short summers because of the adaptable plant density, active N_2 fixation activity, earlier flowering time, and less insect damage. Hairy vetch is recommended for southern regions because of the late flowering nature leading to ineffective control of winter weeds in early spring but effectively control spring weeds in late spring from the greater biomass per plant. White clover is best for spring weed control and it also has greater N_2 fixation and higher plant densities with corresponding biomass yield. However, the dense root system may be a hindrance to initial rice plant establishment in a no-till cropping regime.

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