B-16.1.3 Transportation Potentials of Methane and Nitrous Oxide via Rice Plants

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Total Budget for FY1997 1,527,000 Yen

Abstract Rice plants of different verity were hydrogenically cultivated for testing the methane transportation potential. The growth of rice was followed during the whole vegetation season. The seedling period of all the tested plants were around 30 days. Tillering stage occurred around 70 days. Parameters to describe the plant physiological properties were monitored. Methane emission rate from rice cultivars ranged between 0.6-1.9 umol /min /plant. Difference of methane transportation potential caused by rice verities were found. Methane emission rate is found to be correlated with plant properties, internal volume of stems. Under the present testing condition, photosynthesis was more intensive than plant respiration.

Key Words Global Warming, Methane, Rice, Cultivar, Transport

1. Introduction

Methane emission from rice paddies is one of the major sources which constructed global methane budget[9]. Rice paddy methane is widely believed to be produced in the paddy soils through various microbiological processes and is transported from paddy soil into atmosphere by rice plants[10]. Molecular diffusion at the water-air interface and gas bubble ebullition. Comparing the three major pathways, the flow of methane through aerenchyma system in the rice plants seems to be the most important[4,6,10], while the ebullition of high methane concentration bubbles and diffusion accounted about only 20% of total emitted methane during the entire rice cultivation period[11]. Some studies even indicated that methane emission from rice paddies into atmosphere through rice plant even account 95% of the total methane flux released to the atmosphere through out the entire growin season[4,6,10]. Tremendous efforts have been made to describe the methane transport from the rhizosphere to the atmosphere through rice plant by using some modified diffusion models[12]. Basic parameters required for these models are closely related with the methane concentration of the gas bubbles trapped in the soil and factors describing the physiological status of rice plant[12]. If we assumed that the gases, which is mainly consisted of methane and carbon dioxide, distributed around root mate and the gas phase could to some extent directly contact with the part of the root surface and the stem base of the rice plants. The gas-contact in this way would be the main way for gas releasing from paddy soils to atmosphere. However quantified description of this transportation has not been reported. Another two parameters, temperature of the root zone and pressure of the gas bubbles was taken into consideration by some specific studies[7,8]. Comparing the all these factors concerned, it seems that conductance of rice plants should nevertheless play a rather important role within the whole transportation frame work. The driving force for methane transportation has not been fully elucidated. Widely accepted conception is methane transportation is governed by molecular diffusion[1,3], thermo-osmosis[5], Nouchi[10], studied the mechanisms for methane transportation by rice plant at laboratory condition. According to his results, dissolved methane would first diffuse to the surface water around the rice root. Methane is then gasified in the root cortex and was transported to the shoots by the rice aerenchyma system. Finally methane is released mainly through the micropores located in the lower part of the rice plant. Therefore, with these theories it very natural to conclude that methane emission profile observed should be controlled both by methane production and rice transportation capacity. Some studies have even directly focused the relationship between methane emission rate and biomass of the rice plant, since plant physiological properties are closely related with root exudes, which provide the substrate for methanogens and plant gas conductance which transport produced methane from paddy soil to atmosphere. Based on these considerations, it seems that studies on the influence of physiological properties of rice plant are very helpful in forming a mechanism model which could describe methane emission behavior in the paddy fields. These properties should be mainly determined by rice maturation extend, some parameters closely related with rice verity[13]. Therefore, we conducted this to focus directly on the relationship between methane emission potential and its physical and physiological property and tried to use this these results to explain the seasonal methane emission profile which commonly observed in this field.

2. Method and Measurement

(1) Source of rice cultivar

Rice cultivar altogether 19 verities were collected for experiment. Most of the rice seeds were locally available, two from IRRI(International Rice Research Institute, Philippines) and one from United States. All the seeds for experiment were kept normally at room temperature before hydroponically seedling.

Table 1-1 Rice seeds used for experiment

No.	name	origin	Classified No.	abbreviation
1	ASAHI1	Japan	040412	A1
2	AICHI ASAHI	Japan	920223	AA
3	GINBOUZU	Japan	040671	GB
4	HARUTA ASAHI	Japan	060050	HA
5	IR24	IRRI	140023	IR24
6	IR26	IRRI	140095	IR36
7	KOSHIHIKARI	Japan	921241	KH
8	LEMONT	USA	00059772	LM
9	NOURIN22	Japan	080025	N22
10	NOURIN25	Japan	040640	N25
11	NOURIN3	Japan	040638	N3
12	NOURIN32	Japan	060468	N32
13	NOURIN36	Japan	040641	N36

14	NOURIN37	Japan	920936	N37
15	NIPPONBARE	Japan	050007	NB
16	SHINRIKI	Japan	000118	SR
17	TAMANISHIKI	Japan	040566	TN
18	YUUBABAE	Japan	920989	YB
19	YACHIKOGANE	Japan	920988	YK

Seeds listed in are from Japan, USA, Philippines. Classified No. is given by local agricultural department. Abbreviation is used for following studies.

(2) Seedling and preparation of the rice plant

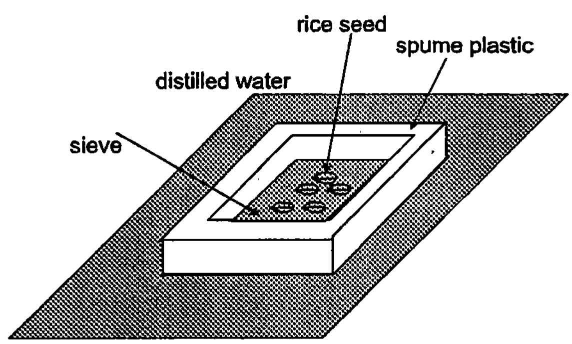

Figure 1-1 The device for seeding the rice. Rice seed was put on the floating net sieve.

The rice was seeded in the distilled water by a floating net sieve in a greenhouse (Figure 2-1). The greenhouse was automatically maintained at 30°C during day time and 20°C during the night time by a temperature program run every 12 hours. Humidity was naturally controlled by the water vaporization in the chamber. Monitored humidity indicated the humidity inside the chamber is around 60-75% of saturated water partial pressure. Sunlight intensity is identical to that of outside chamber. Distilled water was periodically filled to keep the water level. After 27 days, most of the rice seeds were found to germinate and then the rice plant was transplanted into hydroponical plastic chambers (Figure 1-2). The ratio of germination and rice growth condition at the time for transplanting was shown in Table 1-2. Rice from Philippine and USA showed a lower rate of germination and two rice seed from Japan AA and N32 did not show any sign of germination at time when we did the transplanting.

The cultural solution for hydroponically growing the rice is prepared according to Baba[2] with a little modification by taking the experience of previous research workers[10]. The composition and concentration each species were changed with course of maturation of the rice plants to meet the nutrient requirement of the rice at different growing stages. Table 1-3 illustrate the detailed composition of various species used for preparing the cultural solution for rice at different maturation stage.

Table 1-2. Transplanting of the seeded rice and rate of germination

Abbr..	Plant (from)	(to)	Root (from)	(to)	seeded	germinated	rate of germination
(unit)	cm		cm		grain	grain	%
A1	5.5	9.8	3.2	6.5	19	19	100
AA					18	0	0
GB	0.9		6.5		17	13	76
HA	2.2	4.7	4.2	10.2	17	17	100
IR24	2.5	7.3	5.5	22.1	25	18	72
IR36	2	3.6	4	8.6	19	11	58
KH	5.5	9.5	9.8	22.3	23	23	100
LM	1.5	4.5	4.5	7.5	18	17	94
N22	2.5	5	5.5	14.5	17	13	76
N25	2	9.2	6.6	22.6	18	15	83
N3	5	8.5	2.1	7.5	16	16	100
N32					17	0	0
N36	2.3	7.1	4.2	9.3	16	10	63
N37	3.4	10.5	2.3	8.9	15	11	73
NB	3	5.5	5.6	12.2	21	21	100
SR	4.1	8.5	3.4	6	16	16	100
TN	2.3	6.5	5.5	16.5	32	21	66
YB	2.5	6.3	1.4	2.5	20	18	90
YK	0.5	3	2	3.6	21	4	19

Rice come from IRRI, Japan, U.S.A., rice was seeded on Dec.23, 1997. The seeds were placed on thrum net and was seeped in the distilled water. Growing condition was controlled at 20-30C and 54-70% relative humidity. After about three weeks, the rice was transplanted into a hydroponically growing chamber. Rice seeds AA, GB, IR24, IR36, N22, N36,YK showed a rather rate of germination and height of rice plant and length of root are also relatively short.

Table 1-3 Cultural solution used for hydrogenically growing rice at different stage.

Used for 0-6days: chemicals	g/l	used for 7-22 days: chemicals	g/l	used for 22-90 days: chemicals	g/l
DazhongNo1	0.02	$(NH_4)_2SO_4$	0.09636	$(NH_4)_2SO_4$	0.1156
DazhongNo5	0.005	$Ca(NO_3)_24H_2O$	0.1874	$Ca(NO_3)_24H_2O$	0.2250
$Ca(NO_3)_2.4H_2O$	0.01	KNO_3	0.1177	KNO_3	0.1412
$Na_2SiO_3.9H_2O$	0.014	KH_2PO_4	0.0489	KH_2PO_4	0.0588
$MgSO_4.7H_2O$	0.01	K_2SO_4	0.0313	K_2SO_4	0.0345
		KCl	0.0076	KCl	0.0082
		$MgSO_44H_2O$	0.1057	$MgSO_44H_2O$	0.1058
		Fe-EDTA	0.0253	Fe-EDTA	0.0758
		$MnSO_44H_2O$	0.0009	$MnSO_44H_2O$	0.0009
		$ZnSO_47H_2O$	0.0011	$ZnSO_47H_2O$	0.0011

	$CuSO_45H_2O$	0.0003	$CuSO_45H_2O$	0.0003
	$Na_2MoO_42H_2O$	0.0003	$Na_2MoO_42H_2O$	0.0003
	H_3BO_3	0.0136	H_3BO_3	0.0136
	$NaSiO_3$	0.0495	$NaSiO_3$	0.0495
pH=6.5	pH=5.5		pH=5.87	

The aquatic solution for rice growing was specially prepared according to the nutrients necessary for the aquatic rice cultivation(reference, Baba[2)]). During the first 7 days after the transplanting, the aquatic cultural solution was at low concentration. Between 7-22 days, the concentration was increased to normal level. During following days, the concentration of various species were increased a little again. During the whole hydroponically growing period the rice plant was placed under a constant man made condition as was used during seedling period. Experiment on methane transportation ability started on around 38 days and 67 days after the rice was transplanted into the hydroponical chamber(transplanting was conducted on Jan,17,1998). This period is somewhat around the tillering stage and reproduction stage of the rice.

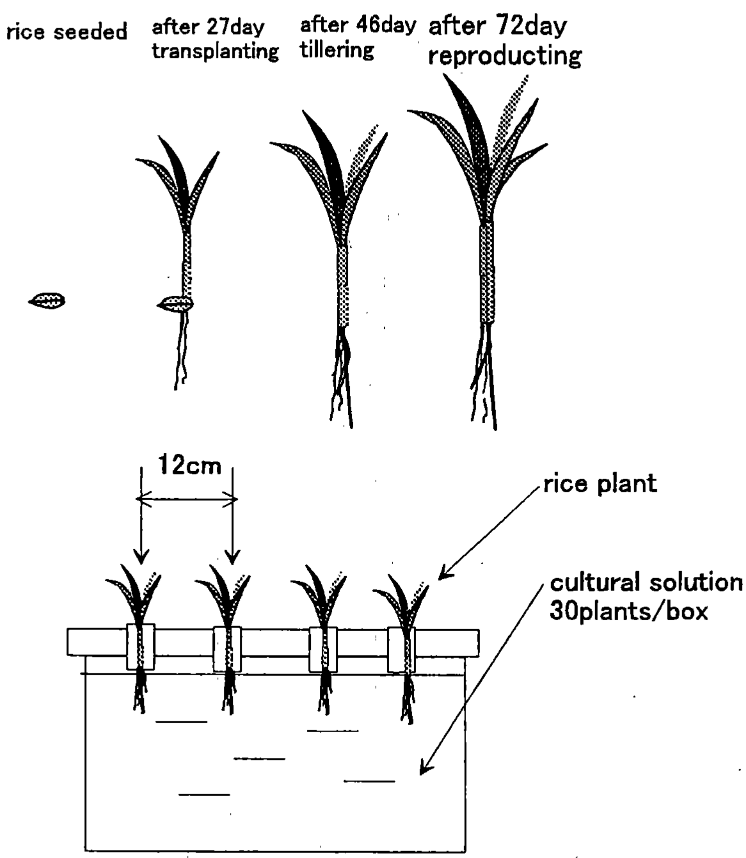

Figure 1-2 The device for hydroponically growing rice. Cultural solution of different concentration was filled into a plastic chamber just reach the rime. Rice plants were insert into small holes(5 in diameter). The plant was supported by soft plastic. The rice roots was shielded by dark paper to prevent it from over intensive sunlight. The cultural solution was taken very 7 days to test root exudes by measuring overall organic carbon content of cultivation solution. Experiments for testing the methane transportation were carried out around 45 and 75 days after the seedling.

(3) Measurement of methane transportation

For testing methane transportation, a PVC static chamber was placed over the plants. The leaves and stems of the plant were properly sealed from surrounding air by module clay. Methane transportation rate was tested by measuring the concentration increase inside the PVC chamber. A small fan was mount at the top of the chamber to make the air inside the chamber well mixed. Before each set of measurement, fresh air was drawn into the chamber to make the methane carbon dioxide concentration inside the chamber return to normal level. Plant roots were seeped in a flask of cultural solution which was prepared according to the composition identical to the cultural solution used for rice growing in greenhouse. replace inside air with replaced by surrounding fresh air (Figure 1-3). The temperature of solution flask was maintained by a constant temperature water bath which was adjusted at 25 C by continuously circulating the water. Cultural solution was saturated by bubbling the pure methane before the experiment. The solution was well mixed during the experiment by running a magnetic stir inside the flask. The methane concentration was found no significant decrease during 1-2 hours measurement[10]. A protecting net was placed over the magnetic stir to prevent injury of the roots caused by magnetic stir.

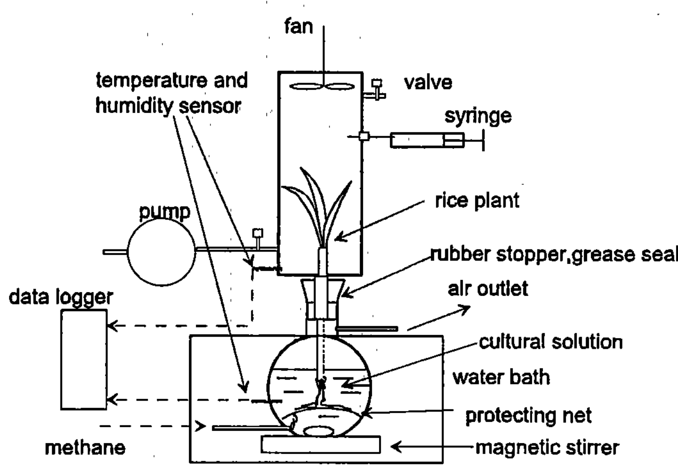

Figure 1-3 Device for testing methane transportation.The methane flux was measured in a closed chamber(PVC,10.8L and 2.32L according to the size of rice plant). The rice plant was held at lower part of the stem by a rubber stopper and sealed with model clay and alginate (which is medically available from the dentists). from surrounding atmosphere.

(4) Measurement of the physiological properties of rice plant.

The monitored physiological parameters of the rice plant include, number of stem, height of rice plant, maximum root length, gas releasing site[10], fresh stem weight, fresh leaf weight, fresh root weight, volume of stem, volume of roots, internal volume of stem, internal volume of roots, leaf area, root total length, stem dry weight, root dry weight, leaf dry weight, stem total length.

Volume of stem was measured by measuring the volume increase caused by seeping the all the rice stem which was cut from leaves and roots into distilled water. The volume of roots internal space was tested by weight difference between vacuum water saturated stem root (surface water was removed) and normal fresh roots. The vacuum water saturated roots was prepared by seeping the fresh roots into the distilled water and then applying the container with high vacuum (10^{-3}mmHg). The air inside the roots will expand and release from the roots and be replaced by distilled water. By using similar way, internal space of stems were also tested. Leaf area was tested by a leaf area scanner (PlantPhy, A-21). Total root length was tested by a commercially available root scanner produced in Finland (RS-A-1).

(5) Measurement of methane and carbon dioxide

Methane was tested by a Shimadzu GC 14A gas-chromatograph. Installed with a Propack Q 80-100mesh packed column (1m.0.5mmid). Detector were FID for testing methane and serially connected TCD for testing CO_2. The whole GC system was controlled by a HP CR-5A Plus computer to automatically change the 2ml injection loop. Methane dissolved in the cultural solution was tested by directly injecting 1ul cultural solution into the GC. Standard gas was provided by Japanese Standard Gas Company Ltd.

3. Results and discussion

(1) Maturation of the rice plants

The development of rice plant during the experiment was showed in Figure 2-1. The growth of rice during first 2-3 weeks are very stable. Dramatic growth of the rice plant occurred at 5th week, which is approximately around the tillering stage. And also lot of old roots were found and simultaneously new germinated very fast. The growth profile of rice, in some extend roughly correlated with methane seasonal variation tendency, indicating that rice maturation growth, to some extend, contributed to the seasonal variation of methane emission rate which was commonly observed by lot of authors doing field experiment[14]. In our study, experiment on methane transportation was carried out around these days.

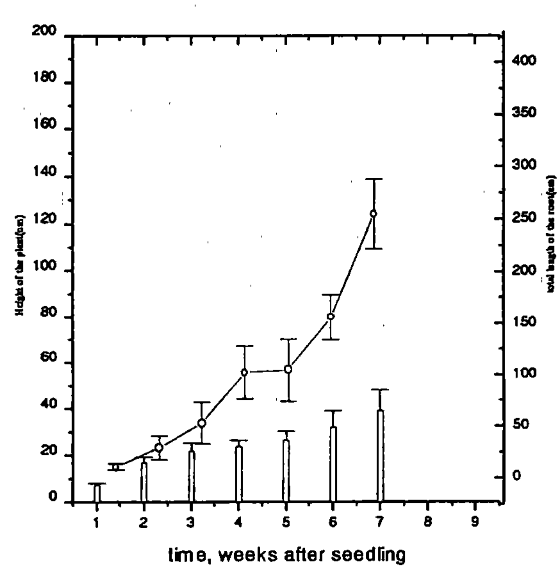

Figure 2-1 Growth of rice during the hydroponically cultivation. The growth condition showed was the condition of SR(Japan), which is typical growth of all the rice plant observed. After 5th week, the root length increase rapidly, while growth during the first a few week is stable.

(2) Time required for making methane saturated solution

Methane concentration in the cultural solution increase exponentially when it was bubbled with pure methane at different flow rate. The highest methane saturate concentration of our cultural solution could reach 1.4-1.6mmole/L. Some higher saturated methane concentration could be found from used cultural solution (used for testing transportation, detailed data was not shown) indicating methane saturated concentration is affected by chemical composition of solution. The higher flow rate cause earlier saturation of the cultural solution (Figure 2-2). During the experiment, the methane flow rate varied between 400-1000ml/min. The methane flow rate at this range is usually used for make methane saturated solution.. The results indicated that normally the time required for making saturated solution should be 6-18 min if methane was bubbled at 400-1000ml/min rate. Especially, higher methane bubbling rate would help in well mixing the solution and also saving pure methane. Since the minimum bubbling time at higher flow rate is 6min, and methane consumption is therefore 6000ml while at lower flow rate, the consumption would be around 7000ml.

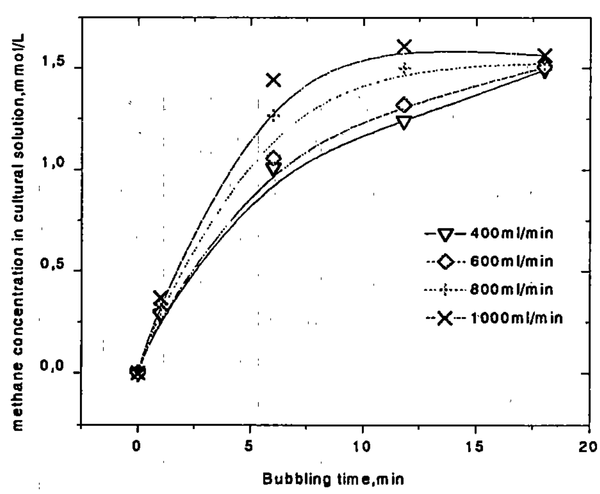

Figure 2-2, The time required for making methane saturated cultural solution. Cultural solution(2250ml) bubbled with pure methane(99.8%). The flow rate was controlled at 400 ml/min, 600 ml/min, 800 ml/min, 1000 ml/min respectively. Temperature was stabilized at 25C. Dissolved methane was measurement by GC-FID. Result indicated, the time required for making saturated methane solution varied between 6-18min depending on different bubbling rate.

(3) Methane transportation rate

Methane concentration inside the static chamber increased linearly once the chamber was sealed from outside atmosphere, while the carbon dioxide concentration inside the chamber decreased simultaneously. The methane increasing rate of different plants varied greatly among the different rice cultivar indicating rice verity is definitely a important factor controlling methane transportation ability. Since the rice plant was exposed to the light (the light intensity is 1.5lux, about 1.98v at standard light sensor), decrease of carbon dioxide could be believed as the result of photosynthesis. However, we do not have detailed knowledge about intensity of the plant respiration, the decreasing of carbon dioxide observed in our study is actually an overall performance of rice plant, rather than isolated photosynthesis rate.

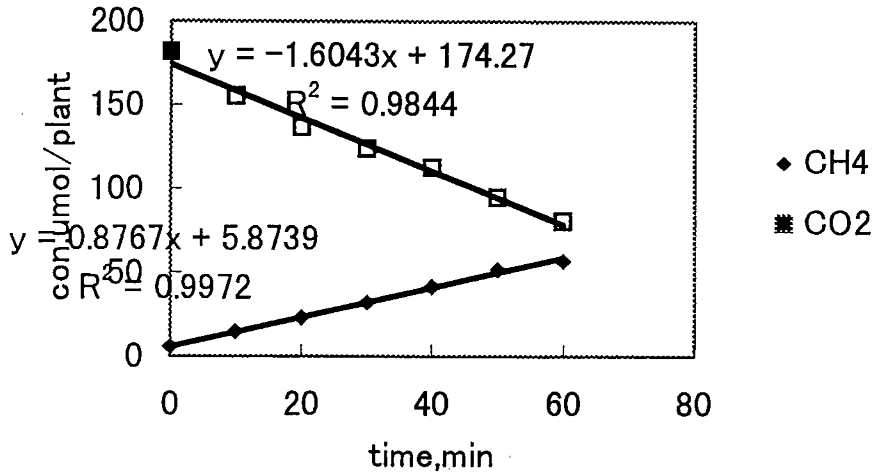

Figure 2-3 Methane emission rate and CO_2 consuming rate. The graph indicated a typical linear variation of methane and CO_2 concentration in the static chamber(SR, Japan 47days after transplanting).

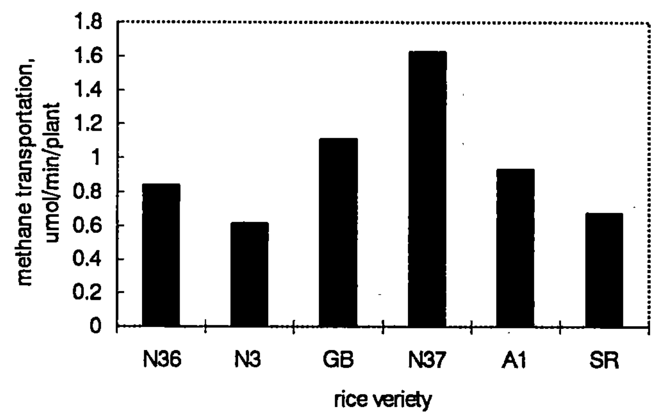

Figure 2-4. Methane transportation rate of different cultivar. The results of SR is the average value of three measurements. Relative error is 12%(P=0.05). Rest are from single measurement.

(4) Difference in rice transportation ability and the relationship with rice plants

Conductance from different rice plant varied greatly(Figure 2-4). N37 indicated a highest emission rate 1.8umol/min/plant. The relationship between methane emission rate and plant properties are not quite clear. The better correlated factors are the volume of stem and stem intercellular volume (Figure 2-5, Table 2-1). Detailed explanation on this results is not available yet. However, these results seem to correspond the previous observations on methane transportation mechanisms[19,8]. In their studies they found most of methane was released at lower part of the stem. And stem is major releasing site of methane.

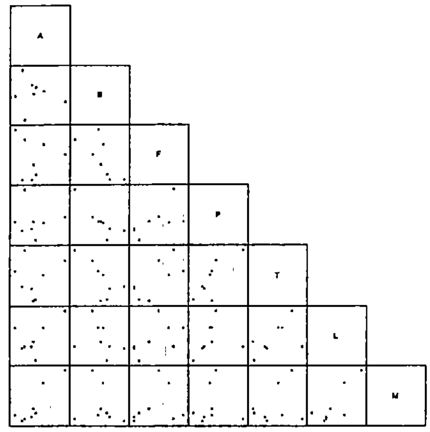

Figure 2-5 Correlation of various tested parameters. Detailed description of each parameters was listed in Table 2-1

Table 2-1 Correlation of the methane emission rate with plant properties

parameters	A	B	F	P	T	L	M
A, Methane emission rate,umol/min/plant	1						
B, CO2 consuming rate, umol/min/plant	-0.152	1					
F, gas releasing site	-0.093	-0.702	1				
P, leaf area,cm^2	-0.049	-0.862	0.629	1			
T, leaf fresh weight, g	0.140	-0.712	0.713	0.810	1		
L, stem volume, cc	0.554	-0.296	0.555	0.254	0.546	1	
M, stem intercellular volume, cc	0.898	-0.198	0.252	0.029	0.303	0.798	1

Besides the correlation with methane emission rate, a negative correlation was found between CO_2 decreasing rate and plant leaf area and also the gas releasing site indicating photosynthesis is, at the moment, dominate physiological activities of the plant.

4. Conclusion

Under hydroponically growing condition, rice plant was found to actively grow during 4-5 weeks after transplanting. Maximum methane saturated concentration of the cultural solution used for experiments are around 1.4-1.6mmole/L. Methane emission rate from different rice cultivar ranged between 0.6-1.9umol/min/plant. Maximum emission rate was found from a plant, N37, Japan. Minimum emission rate exhibited by N3, Japan. Methane emission rate is found to be correlated with properties concerning rice stem. CO_2 decreasing rate is negatively correlated with leaf area and gas releasing site of the plants indicating under the present testing condition, photosynthesis is more intensive than plant respiration.

5. Acknowledgments,

We thank S.Yonemura, H.Sudo K.Hosen, M.Watanabi, W.Ishikawa, for providing us technical assistance. We thank H.Sakai, H.Hesagawa lending us the root scanner and leaf area scanner. We thank also K,Minami,H.Tsuruta for the valuable discussions on details of the research. We especially wish to express our thanks to I.Nouchi for his tremendous help and great care in processes of the experiment. This work is a contribution to Association of International Research Initiatives for Environmental Studies.

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