Chapter II

Materials and Methods

2.1 Materials

2.1.1 Skim latex

Two types of skim latex were used in this research;

2.1.1.1 Skim latex from ordinary process of 60% concentrated latex was obtained from Rayong Bangkok Rubber Co.,Ltd.

2.1.1.2 Deproteinized skim latex was obtained after deproteinization of ammoniated latex with Alcalase enzyme and centrifugation (Boonjawat et al., 2000)

2.1.2 rice seeds

Two cultivars of rice (*Oryza sativa L.*) were kindly provided by Pathumthani Rice Research Center.

2.1.2.1 Khao Dawk Mali 105 (KDML 105)

2.1.2.2 Suphan Buri 1 (SPR 1)

2.1.3 soil

The soil of the three pot experiments was classified as acid sulphate clay soil in Rangsit soil series. The characteristics of soil were analyzed by the Department of Soil Science, Faculty of Agriculture, Kasetsart University and summarized in Table 2.1

Table 2.1 Characteristics of clay soil used in pot experiment

Soil characteristics	Experiment I	Experiment II&III
pH (soil : water 1:1)	4.2	4.6
Texture (hydrometer)	%sand:%silt:%clay 29:20:51	%sand:%silt:%clay 25:18:57
Organic matter (%)	2.4	2.3
Lime requirement	1,613	1,345
Total nitrogen (g%)	0.124	0.129
Available phosphorus (Bray II, ppm)	3	5
Potassium (ppm)	130	185
Calcium (ppm)	1,600	2,120
Magnesium (ppm)	260	340

2.1.4 Hoagland Solution (Hoagland and Arnon, 1938) for rice hydroponics culture

Prepare 100X stock solution as follow:

Stock 1	KNO_3	60.6	g/L
Stock 2	$Ca(NO_3)_2$	65.6	g/L
Stock 3	$MgSO_4$	49.0	g/L
Stock 4	$NH_4H_2PO_4$	34.5	g/L
	Fe-EDTA	0.22	g/L
Stock 5	H_3BO_3	0.286	g/L
	$MnCl_2$	0.181	g/L
	$ZnSO_4$	0.022	g/L
	$CuSO_4$	0.008	g/L
	H_2MoO_4	0.009	g/L

Stock solutions were mixed, using 10 mL of each stock solution and make up volume to 1,000 mL with distilled water. Adjust pH to 6.0 with 1N KOH or 1N H_2SO_4 before use.

2.1.5 Nutrient Agar for soil bacterial culture

Nutrient Broth	8	g/L
Agar	20	g/L

Add 8 g of nutrient broth and 20 g of agar in 1,000 mL of distilled water and then autoclaved.

2.1.6 Chemical fertilizer

2.1.6.1 Ammonium phosphate fertilizer 16-20-0 (National Fertilizer Public Co., Ltd.)

2.1.6.2 N:P_2O_5:K_2O compound 15-15-15 (National Fertilizer Public Co., Ltd.)

2.1.7 Chemicals for analysis of chemical composition in latex serum, plant and soil

Ammonium acetate (AJAX CHEMICALS, Australia)

Ammonium molybdate (Fluka AG, Buchs SG, Switzerland)

Ammoniummetavanadate (CARLO EBRA, Italy)

Anhydrous sodiumsulphate

Barium chloride crystal 20-30 mesh (RIEDEL-DE HAEN AG., Germany)

Bromcresol green (Fluka AG, Buchs SG, Switzerland)

Calcium chloride (MERCK, USA.)

Diethylenetriaminepentaacetic acid (DTPA) (Fluka, Switzerland)

Ethylenediaminetetraacetic acid (EDTA) (Fluka, Switzerland)

Gum arabic from Acacia Tree (SIGMA, USA.)

Methyl red (BDH, England)

Polyvinyl alcohol (BDH, England)

Potassium antimony tartrate (CARLO EBRA, Italy)

Potassium dihydrogen phosphate (MERCK, USA.)

Potassium sulphate (APS Finechem, Australia)

Sodium nitroprusside (RIEDEL-DE HAEN AG, Germany)

Triethanolamine (APS Finechem, Australia)

2.1.8 Apparatus

2.1.8.1 Apparatus for rice planting and harvesting

- pot (20 cm in height and diameter)
- plastic pot (27 and 23 cm in height and diameter, respectively)
- scissors for harvesting
- paper bag
- hot air oven (Memmert B50)
- plant grinding machine (Retsch Type SK1, Germany)

2.1.8.2 Apparatus for chemical analysis of serum, plant and soil

- micro Kjeldahl digestion and distillation apparatus
  - BUCHI Digestion Unit K-424, Switzerland
  - BUCHI Scrubber B-414, Switzerland
  - BUCHI Distillation Unit B-324, Switzerland
- Analytical balance (Mettler Toledo AB204-S, Switzerland)
- Atomic absorption spectrophotometer (VARIAN, SpectrAA 300, Australia)
- Spectrophotometer (JENWAY 6400, England)
- pH meter (PHM61 Radiometer Copenhagen, Denmark)
- Platform shaker (innova 2100 NEW BRUNSWICK SCIENTIFIC, U.S.A.)
- Autoclave (HA-3D Hirayama Mfg.Corp., Japan)
- Incubator (GALLENKAMP, Prime incubator, UK)
- Laminar Flow (ISSCO model HT-123, U.S.A.)

2.2 Methods

2.2.1 Method for Coagulation of skim latex

Skim latex obtained after centrifugation of concentrated latex was transported from the Rayong Bangkok Rubber Co.,Ltd. To coagulate skim latex, 1-1.5% (v/v) of sulphuric acid (50% v/v) was added until the pH was about 4-5. The coagulated skim latex was autoclaved at 121°C for 5 minutes, filtered through filter paper and adjusted pH to 6 with 1N KOH.

The serum obtained was stored at 4°C until use. The chemical composition of each lot of serum was analyzed as described in Appendix A.

- Ammonia: Phenolhypochlorite Method (Solorzano, 1969)
- Total nitrogen content: Kjeldahl method (APHA, AWWA and WPCF, 1992)
- Phosphate: Strickland and Parsons, 1972
- Sulphate: turbidimetric method (APHA, AWWA and WPCF, 1992)
- K, Ca, Mg, Cu, Fe, Mn and Zn were determined using Atomic Absorption Spectrophotometer (AAS) by Scientific and Technological Research Equipment Center (STREC).

2.2.2 Growth of rice seedling in hydroponic culture

Experiment was carried out at Pathumthani Rice Research Center. To investigate the proper concentration of serum suitable for rice growing, rice seeds (SPR 1 and KDML 105) were soaked in water for 24 hours, then planted in a tray, which contain clay soil for 7 days. Roots of seedlings were eluted with tap water then transferred to a 150 mL opaque bottle, which contains 150 mL of nutrient solution. Five concentrations of skim latex serum (Lot no. 000903) and deproteinized latex serum (Lot no. 000803) were used in this experiment, which were 1,3,5,7 and 9%v/v. Hoagland solution (Hoagland and Arnon, 1938) and commercial chemical fertilizer ($N:P_2O_5:K_2O$=15-15-15) 2 g/L were used as control treatments.

2.2.3 Growth of rice in CS and DS under greenhouse condition (Pot Experiment I)

A pot experiment was carried out at Pathumthani Rice Research Center. To investigate the proper concentration and the kind of serum, rice seeds (SPR 1 and KDML 105) were soaked in water for 24 hours, and then planted in the tray, which contain clay soil for 7 days. Seedling was transplanted to each pot (20 cm height; 20 cm internal diameter) which contain 4.5 kg of clay soil classified as acid sulphate clay in Rangsit soil series. Chemical composition of clay soil was shown in Table 2.1. Five concentrations of skim latex serum (Lot no. 001020) and deproteinized latex serum (Lot no. 001027) were used in this experiment, which were 1,3,5,7 and 9%v/v as in CS1 to CS9 and DS1 to DS9, respectively. Treatment C1 was fertilizer free treatment. Inorganic fertilizer in the form of ammonium phosphate ($N:P_2O_5:K_2O$=16-20-0) at the rate 30 kg/rai was used as control treatment. This experiment was carried out in completely randomized design (CRD) with ten replications. Chemical fertilizer was applied at two growth stages of rice, 11 days after germination and at the panicle initiation stage (PI). Serum was applied at three growth stages of rice, 11 days after germination, one month after germination and at the beginning of panicle initiation stage.

Table 2.2 Details of N-fertilizer and latex serum application in Pot Experiment I

Treatment	N-fertilizer			
	1st application (gN/pot)	2nd application (gN/pot)	3rd application (gN/pot)	Total N-fertilizer (gN/pot)
C1	-	-	-	-
C2	0.096	0.096	-	0.192
CS1	0.0818	0.0856	0.0856	0.253
CS3	0.2455	0.2567	0.2567	0.7589
CS5	0.4092	0.4278	0.4278	1.2648
CS7	0.5729	0.5989	0.5989	1.7707
CS9	0.7366	0.7700	0.7700	2.2766
DS1	0.0678	0.0784	0.0784	0.2246
DS3	0.2034	0.2351	0.2351	0.6736
DS5	0.3390	0.3918	0.3918	1.1226
DS7	0.4746	0.5485	0.5485	1.5716
DS9	0.6102	0.7052	0.7052	2.0206

*Each pot contains 4.5 kg soil

2.2.4 Growth of rice supplemented with CS in combination with ammonium phosphate fertilizer under greenhouse condition (Pot Experiment II)

To investigate the proper concentration of serum, rice seeds (SPR 1 and KDML 105) were soaked in water for 24 hours, then planted in the tray, which contain clay soil for 7 days. Three seedlings were transplanted to each plastic pot containing 6.5 kg of Rangsit Series clay soil. Three seedlings were planted in each pot. After ten days, one healthy seedling was selected for the experiment. The experiment consisted of 6 treatments as described in Table 2.3. The details of the treatments in this experiment are listed in Table 2.3. Latex serum lot no. 010705 was used in this study. This experiment was carried out in completely randomized design (CRD) with 10 replications. Inorganic fertilizer in the form of ammonium phosphate ($N:P_2O_5:K_2O$=16-20-0) at the rate 30 kg/rai was used as control. The fertilizer and serum were applied at two growth stages of rice, 21 days after germination and at the beginning of panicle initiation (PI).

Table 2.3 Details of N-fertilizer and latex serum application in Pot Experiment II

Treatment	Rate of fertilizer application kg/rai (gN/pot)	Rate of serum application kgN/rai (gN/pot)	Application
T1 (0)	0	0	-
T2 (100F)	30 (0.1246)	0	21 and 57 DAG
T3 (100S)	0	117 (2.992)	split application at 21 and 57 DAG
T4 (25F+75S)	7.50 (0.0312)	87.75 (2.244)	split application at 21 and 57 DAG
T5 (50F+50S)	15 (0.0623)	58.50 (1.496)	split application at 21 and 57 DAG
T6 (75F+25S)	22.50 (0.0935)	29.25 (0.748)	split application at 21 and 57 DAG

2.2.5 Growth of rice in fixed amount of latex serum (100S), and variable chemical fertilizer under greenhouse condition (Pot Experiment III)

This study was conducted similarly to the above study but the treatments were changed to applying serum (Lot no. 010705) at 2.992 gN/pot added with chemical fertilizer (ammonium phosphate 16-20-0) 0.0125, 0.0312 and 0.0623 gN/pot. The details of treatments were shown in Table 2.4.

Table 2.4 Details of N-fertilizer and latex serum application in Pot Experiment III

Treatment	Rate of serum application kgN/rai (gN/pot)	Rate of fertilizer application kg/rai (gN/pot)	Application
T1 (0)	0	0	-
T2 (100F)	0	30 (0.1246)	21 and 57 DAG
T3 (100S)	117 (2.992)	0	split application at 21 and 57 DAG
T7 (100S+10F)	117 (2.992)	3 (0.0125)	split application at 21 and 57 DAG
T8 (100S+25F)	117 (2.992)	7.50 (0.0312)	split application at 21 and 57 DAG
T9 (100S+75F)	117 (2.992)	15 (0.0623)	split application at 21 and 57 DAG

Pre-harvest data gathering:

- Mean plant height at every 7 days after applying serum and fertilizer. The average plant height, in cm, for each treatment was determined through individual height measurements taken from the soil surface to upper most leaf of plants.
- Tiller count at every 7 days until ripening stage
- Panicles/hill

Data gathering of yield and yield components at harvest:

- Dry weight of stem and roots, in grams: The stem and roots from each pot were dried at 60°C for 72 hours and weighed.
- Grain yield, in grams: The grains from each pot were dried at 60°C for 7 days and weighed.
- 100 Grains weight (g)
- Percentage of filled grain and unfilled grain

2.2.6 Analysis of chemical composition of plant

At maturity, straws and seed were harvested. All plant samples were oven-dried to constant weight at 72°C, ground to pass a 0.5 mm screen, and weighed. Plant samples from each treatment were analyzed for:

- Total nitrogen content: Kjeldahl method (Department of Agriculture, 1993)
- Phosphorus: Vanado molybdophosphoric acid yellow method (Department of Agriculture, 1993)
- Sulphate: turbidimetric method (Department of Agriculture, 1993)
- K, Ca, Mg, Cu, Fe, Mn and Zn were determined using Atomic Absorption Spectrophotometer (AAS) by Scientific and Technological Research Equipment Center (STREC).

2.2.7 Analysis of chemical composition of soil

Soil sampling was done before planting and after harvesting of rice for analyzing chemical properties. Soils from each treatment were analyzed for:

- Total nitrogen: Kjeldahl method (Tassanee, 1977)
- Sulphate: Turbidimetric method (Soil Analysis Handbook of Reference Methods, 1999)
- Available phosphorus: Bray II method
- K, Ca and Mg content: extracted by NH_4OAc and determined by AAS
- Organic matter content (OM): Walkley and Black method
- pH of the soils: soil: water = 1:1
- Cu, Fe, Mn and Zn: extracted by DPTA extraction (Soil Analysis Handbook of Reference Methods, 1999) and determined by AAS at STREC.

Available P, K, Ca, Mg, organic matter content, texture, lime requirement and pH of the soils were analyzed by Department of Soil Science, Faculty of Agriculture, Kasetsart University.

2.2.8 Bacterial Colony Count of Soil (Wistreich and Lechtman, 1988)

After applying serum for one week, soils were sampled once a week for three times. For each treatment, five soil samples were collected and mixed evenly. One gram of mixed soil was drawn and placed into glass vial containing 9 mL of sterile distilled water. Serial dilutions were made to 1/10,000,000 dilution. One hundred microliter of the sample were placed in each nutrient agar plate and spreaded. After being incubated at 37°C for 24 hours, bacterial colonies were counted.