APPENDIX

Appendix A

imple Method for Multiplying *Scutellospora* sp. Spores in Pot Culture

Many methods have been used to establish and multiply living cultures of AM
he method described here utilizes plants grown in 1 kg of autoclaved sand in
h drainage.

cedure:

Scutellospora sp. spores from the rhizosphere soil of host plants were isolated by the wet sieving and decanting technique (Brundrett *et al.*, 1996) for pot culturing. All *Scutellospora* spores were rinsed with sterile water.

A single healthy spore was transferred to the centre of sterilized filter paper (approx. 2.5 m^2) using sterile fine forceps and placed in autoclaved sand, in the middle of a sterile plastic pot that contained basal drainage holes.

- One surface sterilized seed with 70% alcohol, rinsed with sterilized water for five times of *Sorghum bicolor* (L.) was placed on top of the filter paper with the *Scutellospora* spore, and buried to a depth of 2-3 cm.
- Each pot was treated twice daily with 200 ml of nutrient solution based on Yoshida's formula (Yoshida *et al.*, 1976) but with concentration of phosphorus reduced ten times to 0.1 mM (see below).

- Plants were grown for three to four months on the bench in an outdoor screen house consisting of a plastic roof and mesh walls and the shoot was cut off at the surface of the soil.
- To check for spore density, a core (25 mm diameter) was taken and transferred to the lab.
- Each pot containing *Scutellospora* spores was overturned separately onto clean newsprint and the sand and roots mixed thoroughly, then inserted into a pre-labeled zip-loc bag.
- The inoculum was stored in a refrigerator at 4 degrees.

This method of producing monosporic cultures of *Scutellospora* that have big spores that are dark colour and can be observed by eyesight, could be useful for studying symbiosis with host plant by using spores directly to infect the roots of plant. By this method it is possible to multiply one spore of *Scutellospora* to more than 40 spores g^{-1} sand.

Table A-1 Preparation of stock solution (Yoshida *et al.*, 1976)

Element	Reagent (AR grade)	Preparation (g 10^{-1} liters of distilled water)
N	NH_4NO_3	914
P	$NaH_2PO_4.2H_2O$	403
K	K_2SO_4	714
Ca	$CaCl_2$	886
Mg	$MgSO_4.7H_2O$	3240
Mn	$MnCl_2.4H_2O$	15*
Mo	$(NH_4)_6.MO_7O_{24}.4H_2O$	0.74*
B	H_3BO_3	9.34*
Zn	$ZnSO_4.7H_2O$	0.35*
Cu	$CuSO_4.5H_2O$	0.31*
Fe	$FeCl_3.6H_2O$	77*
	Citric acid (monohydrate)	119*

* Dissolve separately: then combine with 500 ml of concentrated H_2SO_4. Make up to 10 liter volume with distilled water.

Table A-2 Preparation of culture solution

Element	Milliliter of stock solution per four liters of culture solution	Concentration of element in nutrient solution in ppm
N	5	40
P	5	1 (recommended as 10)
K	5	40
Ca	5	40
Mg	5	40
Mn*		0.5
Mo*		0.05
B*		0.2
Zn*	5	0.01
Cu*		0.01
Fe*		2
Citric acid*		

* 5 ml of combined stock

Procedure:

For every 4 liters of culture solution to be prepared, add 5 ml of each of stock solution (as set out in table 2) to 1 liter of water in a plastic bucket. For example to fill twenty 4 liter pots, add 100 ml of each of the stock solution to 20 liters of demineralized water in the plastic bucket. Stir the solution after adding each reagent to avoid any precipitation.

References:

Brundrett, M., Bougher, N., Dell. B., Grove, T. and Malajczuk, N. 1996. Working with Mycorrhizas in Forest and Agriculture.ACIAR Monograph. Canberra, Australia.

Yoshida, S., Forno, A.D., Cock, H.J., Gomez, A.K. 1976. Boratory Manual for Physiological Studies of Rice. The International Rice Research Institute. 3rd edition. 62-63.

Appendix B

Nutrient Concentration in Brown Rice

Table B-1 Effects of AM inoculation and phosphorus application on K concentration (%) in brown rice of two rice varieties

P level (kg ha^{-1})	Inoculation	KDML105	Bue Bang	mean
1	AM0	0.188	0.247	0.218 A
	AM1	0.179	0.246	0.213 A
	AM2	0.167	0.218	0.193 B
10	AM0	0.162	0.238	0.200 AB
	AM1	0.158	0.219	0.189 B
	AM2	0.158	0.248	0.203 AB
mean	P1	0.178	0.237	0.208
	P10	0.160	0.235	0.197
mean	AM0	0.175	0.243	0.209
	AM1	0.169	0.233	0.201
	AM2	0.163	0.233	0.198
mean	V	0.169 B	0.236 A	

Effect	V	AM	P	VxAM	VxP	AMxP	VxAMxP
F-test	***	ns	ns	ns	ns	*	ns

Different letters indicate significant differences between S concentration by LSD at $P < 0.05$. * significant at $P < 0.05$, ***$P < 0.001$, ns = not significant $P < 0.05$.

Table B-2 Effects of AM inoculation and phosphorus application on S concentration (%) in brown rice of two rice varieties

P level (kg ha^{-1})	Inoculation	KDML105	Bue Bang	mean
1	AM0	0.135	0.217	0.176
	AM1	0.139	0.258	0.199
	AM2	0.147	0.184	0.165
10	AM0	0.133	0.186	0.160
	AM1	0.133	0.166	0.150
	AM2	0.142	0.246	0.194
mean	P1	0.140	0.220	0.180
	P10	0.136	0.199	0.168
mean	AM0	0.134	0.202	0.168
	AM1	0.136	0.212	0.174
	AM2	0.144	0.215	0.180
mean	V	0.138 B	0.220 A	

Effect	V	AM	P	VxAM	VxP	AMxP	VxAMxP
F-test	***	ns	ns	ns	ns	ns	ns

Different letters indicate significant differences between S concentration by LSD at $P < 0.05$. *** significant at $P < 0.001$, ns = not significant $P < 0.05$.

Table B-3 Effects of AM inoculation and phosphorus application on Mg concentration (%) in brown rice of two rice varieties

P level (kg ha^{-1})	Inoculation	KDML105	Bue Bang	mean
	AM0	0.062	0.054	0.058
1	AM1	0.065	0.055	0.060
	AM2	0.063	0.053	0.058
	AM0	0.069	0.063	0.066
10	AM1	0.068	0.060	0.064
	AM2	0.072	0.067	0.070
mean	P1	0.063	0.054	0.059 B
	P10	0.070	0.063	0.067 A
mean	AM0	0.066	0.059	0.062
	AM1	0.066	0.058	0.062
	AM2	0.068	0.060	0.064
mean	V	0.067 A	0.059 B	

Effect	V	AM	P	VxAM	VxP	AMxP	VxAMxP
F-test	***	ns	***	ns	ns	ns	ns

Different letters indicate significant differences between S concentration by LSD at $P < 0.05$. *** significant at $P < 0.001$, ns = not significant $P < 0.05$.

Table B-4 Effects of AM inoculation and phosphorus application on Cl concentration (%) in brown rice of two rice varieties

P level (kg ha^{-1})	Inoculation	KDML105	Bue Bang	mean
	AM0	0.133	0.125	0.129
1	AM1	0.124	0.128	0.126
	AM2	0.111	0.118	0.115
	AM0	0.114	0.158	0.136
10	AM1	0.114	0.125	0.120
	AM2	0.111	0.124	0.118
mean	P1	0.123	0.124	0.124
	P10	0.113	0.136	0.123
mean	AM0	0.124	0.142	0.132
	AM1	0.119	0.127	0.123
	AM2	0.112	0.121	0.116
mean	V	0.118 B	0.130 A	

Effect	V	AM	P	VxAM	VxP	AMxP	VxAMxP
F-test	*	ns	ns	ns	ns	ns	ns

Different letters indicate significant differences between S concentration by LSD at $P < 0.05$. * significant at $P < 0.05$, ns = not significant $P < 0.05$.

Table B-5 Effects of AM inoculation and phosphorus application on Zn concentration (mg kg^{-1}) in brown rice of two rice varieties

P level (kg ha^{-1})	Inoculation	KDML105	Bue Bang	mean
1	AM0	26.1	40.8	33.4
	AM1	25.6	42.5	34.1
	AM2	25.1	38.7	31.9
10	AM0	27.0	44.9	36.0
	AM1	26.7	43.7	35.2
	AM2	30.8	45.5	38.2
mean	P1	25.6	40.7	33.1 B
	P10	28.2	44.7	36.4 A
mean	AM0	26.6	42.9	34.7
	AM1	26.1	43.1	34.6
	AM2	28.0	42.1	35.0
mean	V	26.9 B	42.7 A	

Effect	V	AM	P	VxAM	VxP	AMxP	VxAMxP
F-test	***	ns	**	ns	ns	ns	ns

Different letters indicate significant differences between S concentration by LSD at $P < 0.05$. ** significant at $P < 0.01$, ***$P < 0.001$, ns = not significant $P < 0.05$.

Table B-6 Effects of AM inoculation and phosphorus application on Mn concentration (mg kg^{-1}) in brown rice of two rice varieties

P level (kg ha^{-1})	Inoculation	KDML105	Bue Bang	mean
1	AM0	32.3	52.8	42.6
	AM1	47.6	64.9	56.2
	AM2	37.5	54.3	45.9
10	AM0	32.4	45.9	39.1
	AM1	34.0	48.4	41.2
	AM2	35.5	45.3	40.4
mean	P1	39.1	57.3	48.2 A
	P10	34.0	46.5	40.2 B
mean	AM0	32.4	49.3	40.8
	AM1	40.8	56.6	48.7
	AM2	36.5	49.8	43.1
mean	V	36.5 B	51.9 A	

Effect	V	AM	P	VxAM	VxP	AMxP	VxAMxP
F-test	***	ns	*	ns	ns	ns	ns

Different letters indicate significant differences between S concentration by LSD at $P < 0.05$. * significant at $P < 0.05$, ***$P < 0.001$, ns = not significant $P < 0.05$.

Appendix C

Nutrient Concentration in Husk

Table C-1 Effects of AM inoculation and phosphorus application on N concentration (%) in husk of two rice varieties

P level (kg ha^{-1})	Inoculation	KDML105	Bue Bang	mean
1	AM0	0.46	0.36	0.41
	AM1	0.41	0.37	0.39
	AM2	0.42	0.43	0.42
10	AM0	0.42	0.38	0.40
	AM1	0.42	0.43	0.43
	AM2	0.42	0.37	0.40
mean	P1	0.43	0.39	0.41
	P10	0.42	0.39	0.41
mean	AM0	0.44	0.37	0.40
	AM1	0.41	0.40	0.41
	AM2	0.42	0.40	0.41
mean	V	0.43 A	0.39 B	

Effect	V	AM	P	VxAM	VxP	AMxP	VxAMxP
F-test	*	ns	ns	ns	ns	ns	ns

Different letters indicate significant differences between S concentration by LSD at $P < 0.05$. * significant at $P < 0.05$, ns = not significant $P < 0.05$.

Table C-2 Effects of AM inoculation and phosphorus application on P concentration (%) in husk of two rice varieties

P level (kg ha^{-1})	Inoculation	KDML105	Bue Bang	mean
	AM0	0.020	0.020	0.020
1	AM1	0.021	0.20	0.021
	AM2	0.020	0.20	0.020
	AM0	0.020	0.20	0.020
10	AM1	0.021	0.20	0.021
	AM2	0.024	0.20	0.022
mean	P1	0.020	0.020	0.020
	P10	0.022	0.020	0.021
mean	AM0	0.020	0.020	0.020
	AM1	0.021	0.020	0.020
	AM2	0.022	0.020	0.021
mean	V	0.021 A	0.020 B	

Effect	V	AM	P	VxAM	VxP	AMxP	VxAMxP
F-test	*	ns	ns	ns	ns	ns	ns

Different letters indicate significant differences between S concentration by LSD at $P < 0.05$. * significant at $P < 0.05$, ns = not significant $P < 0.05$.

Table C-3 Effects of AM inoculation and phosphorus application on K concentration (%) in husk of two rice varieties

P level (kg ha^{-1})	Inoculation	KDML105	Bue Bang	mean
1	AM0	1.00	1.62	1.31
	AM1	1.07	1.55	1.31
	AM2	0.97	1.62	1.30
10	AM0	0.93	1.39	1.16
	AM1	1.01	1.43	1.22
	AM2	0.91	1.46	1.18
mean	P1	1.02	1.60	1.31 A
	P10	0.95	1.40	1.19 B
mean	AM0	0.97	1.51	1.24
	AM1	1.04	1.49	1.24
	AM2	0.94	1.54	1.26
mean	V	0.98 B	1.51 A	

Effect	V	AM	P	VxAM	VxP	AMxP	VxAMxP
F-test	***	ns	*	ns	ns	ns	ns

Different letters indicate significant differences between S concentration by LSD at $P < 0.05$. * significant at $P < 0.05$, ***$P < 0.001$, ns = not significant $P < 0.05$.

Table C-4 Effects of AM inoculation and phosphorus application on Na concentration (%) in husk of two rice varieties

P level (kg ha^{-1})	Inoculation	KDML105	Bue Bang	mean
1	AM0	0.010	0.014	0.012
	AM1	0.010	0.013	0.012
	AM2	0.010	0.010	0.010
10	AM0	0.010	0.016	0.013
	AM1	0.011	0.010	0.010
	AM2	0.014	0.010	0.012
mean	P1	0.010	0.013	0.011
	P10	0.012	0.012	0.012
mean	AM0	0.010ab	0.015a	
	AM1	0.010b	0.012ab	
	AM2	0.012b	0.010b	
mean	V	0.011	0.012	

Effect	V	AM	P	VxAM	VxP	AMxP	VxAMxP
F-test	ns	ns	ns	*	ns	ns	ns

Different letters indicate significant differences between S concentration by LSD at $P < 0.05$. * significant at $P < 0.05$, ns = not significant $P < 0.05$.

Table C-5 Effects of AM inoculation and phosphorus application on Ca concentration (%) in husk of two rice varieties

P level (kg ha^{-1})	Inoculation	KDML105	Bue Bang	mean
1	AM0	0.102	0.162	0.132
	AM1	0.089	0.155	0.122
	AM2	0.105	0.165	0.135
10	AM0	0.103	0.172	0.137
	AM1	0.111	0.169	0.140
	AM2	0.115	0.177	0.146
mean	P1	0.099	0.161	0.130 B
	P10	0.109	0.173	0.141 A
mean	AM0	0.102	0.167	0.135
	AM1	0.100	0.162	0.131
	AM2	0.110	0.171	0.140
mean	V	0.104 B	0.167 A	

Effect	V	AM	P	VxAM	VxP	AMxP	VxAMxP
F-test	***	ns	*	ns	ns	ns	ns

Different letters indicate significant differences between S concentration by LSD at $P < 0.05$. * significant at $P < 0.05$, ***$P < 0.001$, ns = not significant $P < 0.05$.

Table C-6 Effects of AM inoculation and phosphorus application on Mg concentration (%) in husk of two rice varieties

P level (kg ha^{-1})	Inoculation	KDML105	Bue Bang	mean
1	AM0	0.039	0.059	0.049
	AM1	0.033	0.052	0.048
	AM2	0.035	0.062	0.043
10	AM0	0.043	0.066	0.054
	AM1	0.043	0.062	0.054
	AM2	0.041	0.068	0.052
mean	P1	0.036	0.058	0.047 B
	P10	0.043	0.065	0.054 A
mean	AM0	0.041	0.062	0.052
	AM1	0.038	0.057	0.048
	AM2	0.038	0.065	0.051
mean	V	0.039 B	0.061 A	

Effect	V	AM	P	VxAM	VxP	AMxP	VxAMxP
F-test	***	ns	***	ns	ns	ns	ns

Different letters indicate significant differences between S concentration by LSD at $P < 0.05$. *** significant at $P < 0.001$, ns = not significant $P < 0.05$.

Table C-7 Effects of AM inoculation and phosphorus application on Cl concentration (%) in husk of two rice varieties

P level (kg ha^{-1})	Inoculation	KDML105	Bue Bang	mean
1	AM0	0.62	0.58	0.60
	AM1	0.62	0.73	0.68
	AM2	0.56	0.83	0.70
10	AM0	0.59	0.77	0.68
	AM1	0.57	0.76	0.66
	AM2	0.56	0.83	0.70
mean	P1	0.60	0.72	0.66
	P10	0.58	0.79	0.68
mean	AM0	0.60	0.68	0.64
	AM1	0.60	0.75	0.67
	AM2	0.56	0.83	0.70
mean	V	0.59 B	0.75 A	

Effect	V	AM	P	VxAM	VxP	AMxP	VxAMxP
F-test	**	ns	ns	ns	ns	ns	ns

Different letters indicate significant differences between S concentration by LSD at $P < 0.05$. ** significant at $P < 0.01$, ns = not significant $P < 0.05$.

Table C-8 Effects of AM inoculation and phosphorus application on Cu concentration (mg kg^{-1}) in husk of two rice varieties

P level (kg ha^{-1})	Inoculation	KDML105	Bue Bang	mean
1	AM0	4.93	5.87	5.40
	AM1	4.26	7.12	5.69
	AM2	5.82	8.43	7.12
10	AM0	3.47	7.74	5.61
	AM1	4.56	5.00	4.77
	AM2	4.89	6.92	5.90
mean	P1	5.00	7.14	6.07
	P10	4.31	6.54	5.43
mean	AM0	4.20	6.81	5.50
	AM1	4.41	6.05	5.23
	AM2	5.35	7.67	6.51
mean	V	4.65 B	6.84 A	

Effect	V	AM	P	VxAM	VxP	AMxP	VxAMxP
F-test	***	ns	ns	ns	ns	ns	ns

Different letters indicate significant differences between S concentration by LSD at $P < 0.05$. *** significant at $P < 0.001$, ns = not significant $P < 0.05$.

Table C-9 Effects of AM inoculation and phosphorus application on Zn concentration (mg kg^{-1}) in husk of two rice varieties

P level (kg ha^{-1})	Inoculation	KDML105	Bue Bang	mean
1	AM0	31.6	40.9	36.3
	AM1	19.9	36.7	28.3
	AM2	19.2	37.3	28.2
10	AM0	22.2	52.9	37.6
	AM1	24.2	39.1	31.6
	AM2	22.9	44.5	33.7
mean	P1	23.6	38.3	30.9
	P10	23.1	45.5	34.3
mean	AM0	26.9	46.9	36.9
	AM1	22.1	37.9	31.0
	AM2	21.1	40.9	30.0
mean	V	23.4 B	41.9 A	

Effect	V	AM	P	VxAM	VxP	AMxP	VxAMxP
F-test	***	ns	ns	ns	ns	ns	ns

Different letters indicate significant differences between S concentration by LSD at $P < 0.05$. *** significant at $P < 0.001$, ns = not significant $P < 0.05$.

Table C-10 Effects of AM inoculation and phosphorus application on Mn concentration (mg kg^{-1}) in husk of two rice varieties

P level (kg ha^{-1})	Inoculation	KDML105	Bue Bang	mean
1	AM0	447.1	709.6	444.3
	AM1	742.9	892.0	634.7
	AM2	543.1	833.1	512.3
10	AM0	441.6	577.8	643.7
	AM1	526.5	745.0	818.5
	AM2	481.6	618.3	725.7
mean	P1	577.7	811.6	694.6
	P10	483.2	647.0	565.1
mean	AM0	444.3	643.7	544.0
	AM1	634.7	818.5	726.6
	AM2	512.3	725.7	619.0
mean	V	530.4 B	729.3 A	

Effect	V	AM	P	VxAM	VxP	AMxP	VxAMxP
F-test	*	ns	ns	ns	ns	ns	ns

Different letters indicate significant differences between S concentration by LSD at $P < 0.05$. * significant at $P < 0.05$, ns = not significant $P < 0.05$.

Table C-11 Effects of AM inoculation and phosphorus application on Fe concentration (mg kg^{-1}) in husk of two rice varieties

P level (kg ha^{-1})	Inoculation	KDML105	Bue Bang	mean
1	AM0	45.9	49.5	47.7
	AM1	40.1	63.8	51.9
	AM2	45.0	49.3	47.2
10	AM0	43.1	66.6	54.8
	AM1	48.6	52.9	50.8
	AM2	55.6	47.5	51.5
mean	P1	43.7	54.2	48.9
	P10	49.1	55.7	52.4
mean	AM0	44.5	58.1	51.3
	AM1	44.4	58.3	51.3
	AM2	50.3	48.4	49.4
mean	V	46.4	54.9	

Effect	V	AM	P	VxAM	VxP	AMxP	VxAMxP
F-test	ns	ns	ns	ns	ns	ns	ns

Different letters indicate significant differences between S concentration by LSD at $P < 0.05$. ns = not significant $P < 0.05$.

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