APPENDIX

APPENDIX A

Layout of experiment design

REP 1

11 SSC-SJ5-N50	21 CI-NWI-N0
12 SSC-SJ5-N0	22 CI-NWI-N50
13 SSC-NWI-N0	23 CI-SJ5-N0
14 SSC-NWI-N50	24 CI-SJ5-N50

REP 3

31 CI-NWI-N0	41 SSC-SJ5-N50
32 CI-NWI-N50	42 SSC-SJ5-N0
33 CI-SJ5-N50	43 SSC-NWI-N0
34 CI-SJ5-N0	44 SSC-NWI-N50

N

15 SSC-SJ5-N50	25 CI-NWI-N50
16 SSC-SJ5-N0	26 CI-NWI-N0
17 SSC-NWI-N50	27 CI-SJ5-N0
18 SSC-NWI-N0	28 CI-SJ5-N50

REP 2

Note:

SSC: Saturated soil culture;

CI: Conventional irrigation;

SJ5 and NW1: Variieties;

NO: Nill N fertilizer;

N50: 50 kg/ha N fertilizer.

Table B-1-1 Effects of water regimes and fertilizer nitrogen on the accumulation of dry matter (kg/ha) by SJ5 and NW1

Treatment[a]		Growing stage							
		V4	V6	R1	R4	R5	R5.5	R6	R7
		20[b]	25	38	50	57	64	72	84
SJ5	SSC-N0	414	825	2305	3548	4534	6542	8145	7407
	SSC-N50	351	812	2483	3741	5490	6951	8843	7452
	CI-N0	537	898	2747	3796	4672	6368	7925	7284
	CI-N50	484	950	2310	3975	5655	7398	8366	7275
		V4	V6	R1	R3	R5	R5.5	R6	R7
		20	27	32	37	45	53	60	72
NW1	SSC-N0	196	824	1520	2487	3338	4207	4809	5556
	SSC-N50	524	963	1453	2590	3308	4614	5707	6092
	CI-N0	444	1101	1431	2539	3255	3936	4506	4803
	CI-N50	500	1188	1693	2395	3143	3953	4459	4890

a. SSC: Saturated soil culture; CI: Conventional irrigation; N50: 50 kg/ha of nitrogen fertilizer; NO: nill nitrogen fertilizer; NW1 and SJ5 are soybean varieties. These are the same in the other parts of the paper.

b. Days after sowing.

Table B-1-2. Mean square (MS) and probability (P) of ANOVA for shoot dry matter[a]

Source		DF[b]	Growing stage															
			V4		V6		R1		R34[c]		R5		R5.5[d]		R6		R7	
			MS	P	MS	P	MS	P	MS	P	MS	P	MS	P	MS	P	MS	P
Replciate		2	353.9	0.18	2127.1	0.19	3844.8	0.17	5922.5	0.07	11549.5	0.18	11501.5	0.50	2001.3	0.78	18998.0	0.56
Water	(W)	1	1255.9	0.06	1895.3	0.19	662.1	0.45	429.4	0.43	11.8	0.95	1631.4	0.74	18971.0	0.25	19348.0	0.47
Error (a)		2	75.2		513.9		762.7		455.0		2545.2		11709.5		7221.5		24441.0	
Variety	(V)	1	184.7	0.26	1310.4	0.13	52734.0	0.00	95612.0	0.00	200250.0	0.00	417420.0	0.00	713910.0	0.00	243510.0	0.02
W*V		1	17.1	0.71	320.5	0.41	52.7	0.86	1452.3	0.25	1133.8	0.58	5425.5	0.51	2727.9	0.58	10044.0	0.51
Error (b)		4	106.6		372.4		1411.4		798.9		3082.3		10563.8		7592.3		19668.5	
Starter N	(N)	1	108.5	0.13	263.1	0.40	14.8	0.91	413.7	0.46	12101.0	0.01	13018.0	0.04	14830.0	0.03	1547.1	0.75
V*N		1	603.9	0.00	131.2	0.55	774.1	0.43	641.3	0.36	16241.0	0.00	3856.5	0.22	307.8	0.70	1370.9	0.77
W*N		1	99.3	0.15	0.7	0.97	304.2	0.61	255.6	0.56	10.9	0.91	200.6	0.77	5418.3	0.14	1019.9	0.80
W*V*N		1	126.9	0.11	50.9	0.70	3347.4	0.12	202.0	0.60	44.4	0.83	3833.2	0.22	1771.4	0.37	535.3	0.85
Error (c)		8	38.4		329.1		1101.5		685.0		880.9		2215.6		1996.0		14683.8	

a. Data was run in Statistix software; b. DF is the degree of freedom; c. The data at this stage was R4 for SJ5 and R3 for NW1 variety; d. R5.5 indicates the sampling was taken between R5 and R6 stages.

Table B-2-1 Effects of water regimes and fertilizer nitrogen on shoot nitrogen concentration (%) by SJ5 and NW1

Treatment		Growing stage							
		V4	V6	R1	R4	R5	R5.5	R6	R7
		20	25	38	50	57	64	72	84
SJ5	SSC-N0	3.26	2.87	2.24	2.49	2.91	2.93	2.85	3.18
	SSC-N50	3.87	3.01	2.46	2.91	2.87	2.85	2.92	3.07
	CI-N0	3.84	2.91	2.88	2.84	3.05	2.78	2.70	3.19
	CI-N50	4.53	3.33	2.56	2.69	2.82	2.94	2.73	2.81
		V4	V6	R1	R3	R5	R5.5	R6	R7
		20	27	32	37	45	53	60	72
NW1	SSC-N0	3.38	2.74	2.95	2.82	3.33	2.91	3.06	3.11
	SSC-N50	3.74	2.93	2.98	2.83	3.15	2.94	2.92	3.12
	CI-N0	3.68	3.06	3.46	2.78	3.04	2.88	3.02	2.88
	CI-N50	4.74	3.54	3.20	2.73	3.38	2.74	2.91	2.97

Table B-2-2. Mean square (MS) and probability (P) of ANOVA for shoot nitrogen concentration

Source	DF	V4 MS	V4 P	V6 MS	V6 P	R1 MS	R1 P	R34 MS	R34 P	R5 MS	R5 P	R5.5 MS	R5.5 P	R6 MS	R6 P	R7 MS	R7 P
Replciate	2	0.16	0.12	0.06	0.39	0.18	0.32	0.03	0.64	0.10	0.11	0.00	0.73	0.01	0.77	0.02	0.68
Water (W)	1	2.45	0.01	0.63	0.06	0.81	0.09	0.00	0.99	0.00	0.91	0.04	0.13	0.05	0.34	0.15	0.23
Error (a)	2	0.02		0.04		0.09		0.06		0.01		0.01		0.03		0.05	
Variety (V)	1	0.00	0.96	0.01	0.84	2.26	0.02	0.02	0.42	0.59	0.00	0.00	0.92	0.19	0.03	0.01	0.63
W*V	1	0.00	0.95	0.12	0.43	0.00	1.00	0.03	0.30	0.01	0.52	0.01	0.34	0.04	0.25	0.01	0.72
Error (b)	4	0.12		0.17		0.15		0.02		0.02		0.01		0.02		0.04	
Starter N (N)	1	2.78	0.00	0.56	0.04	0.04	0.31	0.02	0.59	0.01	0.62	0.00	0.90	0.01	0.60	0.06	0.46
V*N	1	0.01	0.76	0.01	0.82	0.01	0.65	0.04	0.45	0.06	0.13	0.01	0.49	0.05	0.25	0.14	0.28
W*N	1	0.23	0.08	0.13	0.27	0.26	0.03	0.15	0.16	0.04	0.21	0.00	0.76	0.00	0.98	0.01	0.74
W*V*N	1	0.15	0.15	0.00	0.97	0.02	0.43	0.10	0.23	0.19	0.02	0.06	0.13	0.00	0.77	0.05	0.51
Error (c)	8	0.06		0.09		0.04		0.06		0.02		0.02		0.03		0.10	

Growing stage: V4, V6, R1, R34, R5, R5.5, R6, R7

Table B-3-1 Effects of water regimes and starter nitrogen on shoot total nitrogen content (kg/ha) by SJ5 and NW1

Treatment		Growing stage							
		V4	V6	R1	R4	R5	R5.5	R6	R7
		20	25	38	50	57	64	72	84
SJ5	SSC-N0	13.51	23.70	51.25	88.14	131.90	192.40	232.47	235.6
	SSC-N50	13.71	24.48	61.50	108.22	157.88	197.53	257.83	229.5
	CI-N0	20.77	26.16	78.17	108.60	142.78	176.51	214.41	237.4
	CI-N50	21.95	32.60	59.12	107.23	160.08	217.35	228.89	203.3
		V4	V6	R1	R3	R5	R5.5	R6	R7
		20	27	32	37	45	53	60	72
NW1	SSC-N0	6.46	22.41	44.84	70.22	111.37	122.44	146.82	172.1
	SSC-N50	16.14	28.41	43.74	72.99	104.50	135.91	166.57	189.5
	CI-N0	16.28	33.11	50.04	70.42	99.44	113.20	135.71	139.1
	CI-N50	23.86	42.13	54.21	65.17	106.23	108.35	129.94	144.3

Table B-3-2. Mean square (MS) and probability (P) of ANOVA for shoot total nitrogen

Source	DF	Growing stage															
		V4		V6		R1		R34		R5		R5.5		R6		R7	
		MS	P	MS	P	MS	P	MS	P	MS	P	MS	P	MS	P	MS	P
Replciate	2	0.80	0.15	2.49	0.17	6.07	0.03	4.56	0.32	19.48	0.05	10.18	0.46	1.01	0.74	27.36	0.39
Water (W)	1	4.09	0.03	4.59	0.10	6.06	0.03	0.53	0.67	0.03	0.88	4.04	0.57	33.65	0.08	39.50	0.27
Error (a)	2	0.14		0.52		0.17		2.12		1.11		8.75		2.84		16.96	
Variety (V)	1	0.20	0.37	1.37	0.15	12.27	0.01	66.63	0.00	109.78	0.01	346.33	0.00	471.35	0.00	255.00	0.04
W*V	1	0.02	0.79	0.72	0.27	0.30	0.46	2.75	0.22	2.02	0.50	6.21	0.40	0.00	0.99	10.90	0.56
Error (b)	4	0.20		0.43		0.46		1.31		3.61		7.14		7.80		27.28	
Starter N (N)	1	1.29	0.00	1.85	0.04	0.13	0.77	0.99	0.30	6.99	0.03	11.19	0.07	10.85	0.18	1.17	0.82
V*N	1	0.95	0.00	0.23	0.41	0.54	0.55	1.69	0.19	7.07	0.03	5.24	0.18	2.52	0.50	14.84	0.43
W*N	1	0.00	0.79	0.28	0.37	2.17	0.24	3.25	0.08	0.10	0.77	1.14	0.51	4.97	0.35	6.05	0.61
W*V*N	1	0.03	0.45	0.03	0.78	4.47	0.11	0.67	0.39	1.86	0.22	10.95	0.07	0.81	0.70	0.93	0.84
Error (c)	8	0.05		0.31		1.37		0.82		1.07		2.45		4.99		21.58	

Table B-4-1 Effects of water regimes and starter nitrogen on nodule dry weight (g/plant) by SJ5 and NW1

Treatment		Growing stage							
		V4	V6	R1	R4	R5	R5.5	R6	R7
		20	25	38	50	57	64	72	84
SJ5	SSC-N0	0.0632	0.1237	0.3128	0.3817	0.4871	0.6013	0.3275	0.037
	SSC-N50	0.0597	0.0757	0.2813	0.5034	0.5955	0.4775	0.3683	0.150
	CI-N0	0.0153	0.0600	0.2116	0.3241	0.2260	0.3968	0.2428	0.040
	CI-N50	0.0077	0.0137	0.1380	0.3060	0.2817	0.4205	0.1665	0.029
		V4	V6	R1	R3	R5	R5.5	R6	R7
		20	27	32	37	45	53	60	72
NW1	SSC-N0	0.0723	0.1223	0.3017	0.3028	0.4243	0.5909	0.3013	0.200
	SSC-N50	0.0567	0.1073	0.3240	0.3222	0.4034	0.4639	0.6367	0.313
	CI-N0	0.0320	0.0713	0.2108	0.2097	0.2301	0.2773	0.1068	0.082
	CI-N50	0.0123	0.0290	0.1670	0.1719	0.1666	0.2391	0.1947	0.116

Table B-4-2. Mean square (MS) and probability (P) of ANOVA for nodule dry weight

Source	DF	V4 MS	V4 P	V6 MS	V6 P	R1 MS	R1 P	R34 MS	R34 P	R5 MS	R5 P	R5.5 MS	R5.5 P	R6 MS	R6 P	R7 MS	R7 P
Replicate	2	0.05	0.53	0.01	0.51	1.39	0.11	1.59	0.49	8.81	0.01	2.67	0.01	2.02	0.54	1.72	0.46
WAter (W)	1	1.14	0.04	2.44	0.01	9.10	0.02	11.96	0.11	37.93	0.00	23.98	0.00	44.39	0.05	7.01	0.16
Error(a)	2	0.05		0.02		0.16		1.56		0.13		0.02		2.38		1.50	
Varirety (V)	1	0.05	0.31	0.12	0.39	0.14	0.73	7.33	0.06	5.01	0.06	3.96	0.06	1.08	0.06	7.79	0.04
W*V	1	0.00	0.88	0.00	0.95	0.00	0.98	0.23	0.68	0.77	0.37	2.88	0.09	3.71	0.01	1.46	0.27
Error(b)	4	0.04		0.13		1.01		1.15		0.75		0.60		0.16		0.91	
Starter N (N)	1	0.12	0.00	0.86	0.00	0.60	0.06	0.87	0.16	0.24	0.58	2.65	0.33	3.07	0.00	2.33	0.01
V*N	1	0.01	0.20	0.05	0.24	0.26	0.18	1.33	0.09	2.31	0.11	0.16	0.81	6.72	0.00	0.08	0.52
W*N	1	0.00	0.87	0.02	0.40	0.44	0.10	0.63	0.22	0.34	0.51	2.11	0.38	1.51	0.02	1.54	0.02
W*V*N	1	0.00	0.37	0.03	0.35	0.02	0.69	0.01	0.88	0.00	0.94	0.12	0.83	1.03	0.05	0.08	0.51
Error(c)	8	0.00		0.03		0.12		0.36		0.71		2.44		0.19		0.17	

Table B-5-1 Effects of water regimes and starter nitrogen on nodule number (nodules/plant) by SJ5 and NW1

Treatment		Growing stage							
		V4	V6	R1	R4	R5	R5.5	R6	R7
		20	25	38	50	57	64	72	84
SJ5	SSC-N0	37.50	43.23	86.72	79.89	85.62	72.19	39.27	4.19
	SSC-N50	32.70	40.93	88.27	98.63	106.19	71.34	41.30	16.17
	CI-N0	23.07	35.97	76.58	59.67	38.09	53.33	29.72	4.56
	CI-N50	18.87	23.60	65.41	72.56	61.00	64.11	24.60	1.69
		V4	V6	R1	R3	R5	R5.5	R6	R7
		20	27	32	37	45	53	60	72
NW1	SSC-N0	46.90	71.83	84.20	113.30	125.07	122.18	65.27	30.11
	SSC-N50	38.80	59.13	86.60	98.40	100.06	96.64	118.09	41.40
	CI-N0	29.83	42.77	65.73	74.87	62.21	59.00	21.80	13.00
	CI-N50	25.10	33.70	57.43	77.97	54.11	54.13	30.70	13.33

Table B-5-2. Mean sqaure (MS) and probability (P) of ANOVA for nodule number

Source	DF	Growing stage															
		V4		V6		R1		R34		R5		R5.5		R6		R7	
		MS	P	MS	P	MS	P	MS	P	MS	P	MS	P	MS	P	MS	P
Replciate	2	49.8	0.26	44.7	0.60	992.5	0.09	1577.6	0.27	4542.9	0.04	1262.8	0.02	703.8	0.62	231.2	0.45
Water (W)	1	1306.9	0.01	2346.3	0.03	3164.1	0.03	4146.2	0.12	15232.0	0.01	6511.9	0.00	9256.3	0.10	1318.2	0.12
Error (a)	2	17.2		66.2		96.8		592.2		174.5		21.5		1136.3		187.6	
Variety (V)	1	304.6	0.19	1521.6	0.00	848.4	0.36	1085.3	0.12	957.9	0.11	1889.5	0.02	3923.6	0.02	1903.0	0.01
W*V	1	2.3	0.90	335.3	0.02	250.8	0.60	59.3	0.67	97.0	0.55	2375.5	0.01	4103.7	0.01	361.9	0.11
Error (b)	4	120.1		23.9		792.0		278.8		229.3		128.8		244.2		83.6	
Starter N (N)	1	178.8	0.02	497.8	0.02	172.2	0.15	147.4	0.37	40.3	0.75	157.2	0.56	1289.6	0.04	161.3	0.24
V*N	1	5.5	0.61	18.9	0.57	1.8	0.87	707.5	0.07	2200.0	0.04	610.3	0.27	1575.1	0.03	2.4	0.88
W*N	1	5.9	0.60	15.5	0.60	10.4	0.70	55.4	0.58	139.1	0.55	391.2	0.37	978.3	0.06	249.4	0.16
W*V*N	1	2.9	0.71	70.4	0.28	353.7	0.05	213.1	0.29	79.7	0.65	30.8	0.80	507.2	0.16	5.6	0.82
Error (c)	8	19.8		53.2		67.4		164.3		363.6		429.4		211.0		101.3	

Table B-6-1 Effects of water regimes and fertilizer nitrogen on nodule unit weight (mg/nodule) by SJ5 and NW1

	Treatment	Growing stage							
		V4	V6	R1	R4	R5	R5.5	R6	R7
		20	25	38	50	57	64	72	84
SJ5	SSC-N0	1.69	2.88	3.65	4.83	6.10	8.33	10.02	7.35
	SSC-N50	1.53	1.93	3.18	5.07	5.74	6.83	9.05	10.06
	CI-N0	0.66	1.66	2.78	4.46	5.98	7.54	7.33	9.64
	CI-N50	0.39	0.55	2.08	4.21	4.33	6.52	6.93	10.64
		V4	V6	R1	R3	R5	R5.5	R6	R7
		20	27	32	37	45	53	60	72
NW1	SSC-N0	1.51	1.68	2.63	2.63	3.41	4.72	11.46	7.01
	SSC-N50	1.47	1.81	3.28	3.25	4.03	4.80	5.41	7.34
	CI-N0	1.04	1.67	2.72	2.70	3.60	4.68	4.90	6.37
	CI-N50	0.48	0.82	2.05	2.12	3.02	4.18	6.44	8.54

Table B-6-2. Mean sqaure (MS) and probability (P) of ANOVA for nodule unit weight

Source	DF	V4		V6		R1		R34		R5		R5.5		R6		R7	
		Growing stage															
		MS	P	MS	P	MS	P	MS	P	MS	P	MS	P	MS	P	MS	P
Replciate	2	0.09	0.79	0.15	0.02	0.19	0.64	1.05	0.10	0.98	0.71	0.25	0.42	10.11	0.66	13.09	0.02
Water (W)	1	4.92	0.06	4.88	0.00	3.63	0.08	1.97	0.56	2.07	0.45	1.17	0.13	40.12	0.29	4.31	0.05
Error (a)	2	0.34		0.00		0.34		0.12		2.41		0.19		19.45		0.23	
Variety (V)	1	0.02	0.71	0.40	0.35	0.39	0.20	23.17	0.01	24.44	0.01	44.15	0.00	9.87	0.58	26.40	0.10
W*V	1	0.20	0.29	0.96	0.17	0.26	0.27	0.01	0.93	0.18	0.70	0.07	0.78	0.19	0.94	1.94	0.60
Error (b)	4	0.13		0.35		0.16		1.17		1.04		0.75		27.24		6.00	
Starter N (N)	1	0.40	0.04	2.88	0.02	0.53	0.08	0.00	0.96	1.46	0.02	3.22	0.13	12.98	0.42	14.37	0.55
V*N	1	0.01	0.71	0.67	0.19	0.49	0.09	0.00	0.96	1.57	0.02	1.65	0.26	3.72	0.66	0.51	0.91
W*N	1	0.15	0.18	0.49	0.26	0.91	0.03	1.08	0.06	2.31	0.01	0.00	0.95	24.91	0.27	0.00	0.99
W*V*N	1	0.06	0.37	0.24	0.42	0.46	0.10	0.10	0.96	0.00	0.91	0.42	0.55	18.53	0.34	4.82	0.72
Error (c)	8	0.07		0.32		0.14		0.11		0.19		1.10		17.91		36.00	

Table B-7-1 Effects of water regimes and starter nitrogen on relative ureide (%) by SJ5 and NW1

Treatment		Growing stage						
		V6	R1	R4	R5	R5.5	R6	R7
		25	38	50	57	64	72	84
SJ5	SSC-N0	45.78	76.78	73.82	83.16	74.28	58.66	42.96
	SSC-N50	11.45	66.25	65.69	83.46	72.26	64.76	41.20
	CI-N0	30.38	51.44	78.42	78.69	56.91	55.17	40.49
	CI-N50	2.01	48.89	74.39	74.83	61.98	51.62	26.66
		V6	R1	R3	R5	R5.5	R6	R7
		27	32	37	45	53	60	72
NW1	SSC-N0	38.69	73.32	55.03	81.59	73.20	60.06	68.66
	SSC-N50	16.99	61.73	50.68	75.51	68.90	67.15	66.91
	CI-N0	24.04	53.69	45.78	83.16	65.52	66.27	42.08
	CI-N50	2.27	38.39	39.94	78.09	69.23	70.81	51.07

Table B-7-2. Mean square (MS) and probability (P) of ANOVA for relative ureide

Source	DF	Growing stage													
		V6		R1		R34		R5		R5.5		R6		R7	
		MS	P	MS	P	MS	P	MS	P	MS	P	MS	P	MS	P
Replicate	2	212.69	0.26	925.40	0.03	41.38	0.50	74.46	0.46	321.01	0.32	32.92	0.54	16.33	0.87
Water (W)	1	1102.70	0.06	2750.80	0.01	16.80	0.59	29.97	0.56	459.64	0.22	16.62	0.58	1324.20	0.08
Error (a)	2	74.82		26.87		41.63		63.46		152.62		39.39		111.94	
Variety (V)	1	21.81	0.23	98.74	0.33	3816.80	0.01	1.19	0.90	48.82	0.40	438.19	0.01	2246.70	0.07
W*V	1	7.71	0.45	0.03	0.99	415.17	0.22	111.80	0.25	154.58	0.17	261.16	0.02	242.13	0.47
Error (b)	4	10.97		81.21		196.03		63.06		55.75		10.00		375.38	
Starter N (N)	1	4227.30	0.00	599.40	0.01	187.38	0.04	81.25	0.22	2.26	0.85	76.43	0.20	26.19	0.59
V*N	1	138.62	0.08	71.69	0.31	1.46	0.83	21.55	0.51	4.98	0.78	30.22	0.41	195.68	0.16
W*N	1	12.97	0.56	6.89	0.74	2.55	0.78	3.73	0.78	85.47	0.26	56.77	0.26	0.66	0.93
W*V*N	1	13.62	0.55	51.28	0.38	11.76	0.55	10.04	0.65	0.32	0.94	19.57	0.50	195.45	0.16
Error (c)	8	35.20		59.71		30.64		46.09		57.67		39.40		81.19	

Table B-8-1 Effects of water regimes and starter nitrogen on the proportion of nitrogen fixed (%) by SJ5 and NW1

	Treatment	Growing stage						
		0-V6	V6-R1	R1-R4	R4-R5	R5-R5.5	R5.5-R6	R6-R7
		0-25	25-38	38-50	50-57	57-64	64-72	72-84
SJ5	SSC-N0	24.59	67.77	82.58	84.93	85.28	67.67	44.19
	SSC-N50	3.99	40.86	70.16	79.91	84.84	70.81	47.52
	CI-N0	15.35	43.33	70.82	84.91	68.78	52.11	39.80
	CI-N50	0.00	26.46	66.27	79.96	70.65	53.24	29.37
		0-V6	V6-R1	R1-R3	R3-R5	R5-R5.5	R5.5-R6	R6-R7
		0-27	27-32	32-37	37-45	45-53	53-60	60-72
NW1	SSC-N0	20.34	61.45	66.41	70.51	84.14	68.00	64.59
	SSC-N50	7.32	41.47	56.19	62.69	76.36	70.08	68.59
	CI-N0	11.54	40.88	47.70	64.76	79.56	66.89	49.78
	CI-N50	0.40	20.55	34.13	56.57	78.54	73.08	59.46

Table B-8-2. Mean square (MS) and probability (P) of ANOVA for proportion of nitrogen fixed

Source	DF	Growing stage													
		V6		R1		R34		R5		R5.5		R6		R7	
		MS	P	MS	P	MS	P	MS	P	MS	P	MS	P	MS	P
Replciate	2	63.16	0.24	685.35	0.07	457.80	0.07	24.60	0.46	392.76	0.25	235.96	0.17	5.90	0.93
Water (W)	1	314.00	0.06	2420.00	0.02	1193.80	0.03	52.66	0.25	410.27	0.21	366.21	0.11	807.36	0.09
Error (a)	2	19.89		52.17		33.86		20.65		127.48		48.46		82.82	
Variety (V)	1	7.01	0.24	74.20	0.24	2735.60	0.01	2119.30	0.01	30.76	0.55	438.53	0.04	2499.00	0.03
W*V	1	2.31	0.47	2.61	0.81	236.69	0.28	53.07	0.45	300.26	0.11	459.64	0.03	0.65	0.96
Error (b)	4	3.61		39.14		153.44		74.36		71.89		46.23		249.74	
Starter N (N)	1	1355.00	0.00	2651.90	0.00	622.71	0.01	252.92	0.03	20.33	0.51	59.06	0.17	15.78	0.58
V*N	1	52.07	0.11	4.54	0.78	17.39	0.56	13.64	0.56	39.25	0.36	6.03	0.64	160.48	0.10
W*N	1	18.99	0.31	35.23	0.45	7.67	0.70	0.03	0.98	30.90	0.42	1.65	0.81	24.97	0.48
W*V*N	1	4.28	0.62	40.46	0.42	47.07	0.35	0.07	0.97	7.43	0.69	14.03	0.48	140.55	0.12
Error (c)	8	16.34		56.28		47.71		36.45		41.98		25.55		46.51	

Table B-9-1 Effects of water regimes and fertilizer nitrogen on cumulative amounts of nitrogen fixed (kg/ha) by SJ5 and NW1

Treatment		Growing stage						
		V6	R1	R4	R5	R5.5	R6	R7
		25	38	50	57	64	72	84
SJ5	SSC-N0	5.00	23.99	54.50	91.59	144.93	172.16	174.03
	SSC-N50	0.84	15.43	48.39	89.49	124.83	165.89	150.67
	CI-N0	3.39	24.80	45.85	74.31	98.51	118.72	127.45
	CI-N50	0.00	6.68	38.75	80.09	124.28	131.04	123.39
		V6	R1	R3	R5	R5.5	R6	R7
		27	32	37	45	53	60	72
NW1	SSC-N0	3.53	17.92	35.18	64.05	73.65	89.48	106.13
	SSC-N50	1.56	7.47	24.42	43.70	68.18	88.13	105.38
	CI-N0	3.01	10.12	20.05	38.82	49.91	65.02	67.60
	CI-N50	0.16	2.72	6.39	29.64	31.11	46.64	54.96

Table B-9-2. Mean sqaure (MS) and probability (P) of ANOVA for amount of nitrogen fixed

Source	DF	Growing stage													
		V6		R1		R34		R5		R5.5		R6		R7	
		MS	P	MS	P	MS	P	MS	P	MS	P	MS	P	MS	P
Replciate	2	0.00	0.90	0.27	0.27	1.90	0.38	0.13	0.69	25.51	0.16	18.28	0.09	42.28	0.08
Water (W)	1	0.07	0.12	1.58	0.06	9.89	0.10	16.30	0.02	43.61	0.09	89.17	0.02	99.39	0.04
Error (a)	2	0.01		0.10		1.17		0.30		4.70		1.83		3.89	
Variety (V)	1	0.00	0.18	4.02	0.01	38.58	0.00	995.04	0.00	272.90	0.01	334.21	0.00	218.41	0.02
W*V	1	0.00	0.44	0.08	0.53	0.83	0.22	0.60	0.67	0.72	0.80	1.88	0.68	0.87	0.82
Error (b)	4	0.00		0.17		0.39		2.75		10.21		9.82		15.25	
Starter N (N)	1	0.57	0.00	7.41	0.00	5.31	0.01	2.50	0.26	1.29	0.57	0.70	0.48	6.24	0.20
V*N	1	0.03	0.04	0.29	0.44	0.47	0.35	4.15	0.16	3.35	0.36	2.51	0.20	0.74	0.64
W*N	1	0.00	0.93	0.16	0.56	0.06	0.74	1.35	0.40	4.00	0.32	0.01	0.94	0.21	0.80
W*V*N	1	0.01	0.18	0.60	0.27	0.01	0.88	0.04	0.88	13.19	0.09	4.75	0.09	3.65	0.32
Error (c)	8	0.00		0.43		0.48		1.70		3.59		1.30		3.19	

Table B-10. Mean square (MS) and probability (P) of ANOVA for yield and yield components

Source	DF	Yield components and yield											
		Height (cm)		Nodes/ plant		Pods/ node		Seeds/ pod		g/100 seeds		Y-measured kg/ha	
		MS	p	MS	p	MS	p	MS	p	MS	p	MS	p
Replicate	2	246.98	0.08	0.63	0.66	2.48	0.31	0.02	0.05	481.54	0.11	439880	0.04
Water (W)	1	3.60	0.72	0.33	0.66	0.14	0.76	0.01	0.10	100.04	0.33	63	0.21
Error (a)	2	21.95		1.22		1.09		0.00		60.79		19483	
Variety (V)	1	5787.70	0.00	8.88	0.00	43.74	0.00	0.01	0.05	1426.00	0.02	959040	0.05
W*V	1	43.47	0.25	0.00	0.10	0.01	0.91	0.00	0.74	1027.00	0.03	774	0.94
Error (b)	4	24.25		0.11		0.42		0.00		97.42		126380	
Starter N (N)	1	83.25	0.24	0.67	0.10	0.38	0.39	0.02	0.06	672.04	0.05	336	0.10
V*N	1	61.02	0.31	0.01	0.86	0.06	0.72	0.00	0.38	513.37	0.08	5457	0.82
W*N	1	2.87	0.82	0.28	0.27	0.88	0.20	0.00	0.60	672.04	0.05	36633	0.56
W*V*N	1	30.60	0.46	0.20	0.34	0.24	0.48	0.00	0.88	1.04	0.93	141220	0.26
Error (c)	8	51.90		0.20		0.44		0.00		131.62		97062	

Table B-11. Mean square (MS) and probability (P) of ANOVA for nitrogen balance

Source	DF	Trial					
		N-seed (kg/ha)		N-fixed (%)		N-balance (kg/ha)	
		MS	P	MS	P	MS	P
Replciate	2	0.0403	0.77	0.0433	0.14	376.94	0.45
Water (W)	1	0.0004	0.96	0.1247	0.05	6863.10	0.04
Error (a)	2	0.1326		0.0070		304.29	
Variety (V)	1	1.48.1	0.02	0.0876	0.00	3179.80	0.13
W*V	1	0.0451	0.53	0.0001	0.70	236.07	0.63
Error (b)	4	0.0965		0.0006		871.63	
Starter N (N)	1	0.0043	0.71	0.0198	0.10	4469.80	0.02
V*N	1	0.0008	0.87	0.0084	0.26	26.73	0.82
W*N	1	0.0131	0.51	0.0077	0.28	362.00	0.42
W*V*N	1	0.0104	0.56	0.0222	0.08	79.32	0.70
Error (c)	8	0.0279		0.0057		495.29	

Table B-12. Probability of ANOVA considered time as a factor[a]

Source	DF	Trial[b]						
		SHDM	NC	TN	NDW	NUM	PROP	FN
Replciates	2	0.54	0.06	0.32	0.19	0.17	0.12	0.09
Water (W)	1	0.69	0.02	0.56	0.02	0.02	0.03	0.02
Error (a)	2							
Variety (V)	1	0.00	0.02	0.00	0.31	0.00	0.34	0.00
W*V	1	0.35	0.88	0.35	0.13	0.01	0.71	0.83
Error (b)	4							
Starter N (N)	1	0.02	0.04	0.10	0.58	0.86	0.00	0.02
V*N	1	0.38	0.84	0.78	0.81	0.34	0.60	0.32
W*N	1	0.36	0.92	0.35	0.11	0.68	0.46	0.50
W*V*N	1	0.46	0.12	0.91	0.48	0.76	0.89	0.14
Error (c)	8							
Time (T)	7	0.00	0.00	0.00	0.00	0.00	0.00	0.00
N*T	7	0.65	0.00	0.78	0.03	0.18	0.00	0.96
V*T	7	0.00	0.00	0.00	0.00	0.56	0.00	0.00
W*T	7	0.18	0.00	0.01	0.00	0.00	0.01	0.00
V*N*T	7	0.58	0.56	0.43	0.02	0.01	0.61	0.79
W*N*T	7	0.99	0.03	0.90	0.31	0.32	0.96	0.95
W*V*T	7	0.89	0.77	0.88	0.12	0.06	0.01	0.95
W*V*N*T	7	0.94	0.47	0.75	0.97	0.83	0.66	0.47
Error (d)	112							

a. Time factor is considered as a sub-sub-subplot level in this split desighn.

b. SHDM: shoot dry matter; NC: nitrogen concentration; TN: total nitrogen; NDW: nodule dry weight; NOM: nodule number; PROP: proportion of nitrogen fixed; FN: amount of nitrogen fixed.

APPENDIX C

Methods of ureide, nitrate and amino analysis

The analysis of N-solutes in xylem exudates

1 Total ureides

[Reference:Young and Conway, 1942]

1.1 Reagents

A. 0.5 M NaOH

NaOH (analytical grade)	20 g
Distilled water	1 L

B. phenylhydrazine hydrochloride

Phenylhydrazine hydrochloride	0.33 g
Distilled water	100 mL

(To be made fresh on each day of analysis. Keep flask containing stock solution covered with tin-foil once prepared. Store with desiccant in freezer.)

C. 0.65 M HCl

(32% w/w) concentrated hydrochloric acid	6.5 mL
Diluted with distilled water to	100 mL

D. Potassium ferricyanide

Potassium ferricynide	0.833 g
Distilled water	50 mL

(To be made fresh on each day of analysis. Keep flask containing stock solution covered with tin-foil once prepared.)

E. Concentrated hydrochloric acid (32% w/w = 10 M)

F. Allantoin standards

Prepare freshly for each day's analyses a 1 umole/mL allantoin stock solution

Allantoin (store with desiccant)	15.8 mg
Distilled water	100 mL

Dilute stock—

0.1 mL to 10 mL with distilled water (10 nmole/mL), 2.5 mL sample for analysis = 25 nmole

0.2 mL to 10 mL with distilled water (20 nmole/mL), 2.5 mL sample for analysis = 50 nmole

0.4 mL to 10 mL with distilled water (40 nmole/mL), 2.5 mL sample for analysis = 100 nmole

0.6 mL to 10 mL with distilled water (60 nmole/mL), 2.5 mL sample for analysis = 150 nmole

0.8 mL to 10 mL with distilled water (80 nmole/mL), 2.5 mL sample for analysis = 200 nmole

Note: always include 2.5 mL distilled water blanks with standards during analysis. Before proceeding with the ureide assay, a full set of standards should be run through (0-250 nmole) to check linearity of response.

1.2 Procedure

Since colour development is not stable, it is advisable to analyze ureides in batches of 20-30 samples including 2 water blanks and at least 3 ureide standards.

(a) Place 0.2 mL of hot water tissue extract or 0.05-0.1 mL xylem exudate sample into each test tube and dilute to 2.5 mL with distilled water. (Use 2.5 mL of each ureide standard and 2.5 mL for water blanks.)

(b) Add 0.5 mL of 0.5 M sodium hydroxide

(c) Mix and place tubes in a boiling water-bath for 10-15 minutes

(d) Remove tubes and cool to room temperature, then add 0.5

mL of 0.65 M HCl and 0.5 mL of phenylhydrazine solution to each tube.

(e) Mix and place tubes in a boiling water-bath for 2-4 minutes.

(f) Remove from boiling water-bath and immediately plunge tubes into an ice-bath for 15 minutes. The rapidity of cooling is an important factor in development of final colour. If possible an ice-salt mixture should be used. (The reaction may be left at this stage for a short time if necessary.)

(g) Remove from ice bath and add 2 mL of concentrated HCl (also chilled to 0 C) and 0.5 mL potassium frricyanide.

(h) Mix immediately after each addition of potassium ferricyanide.

(i) Read optical density (absorbance) at 525 nm on a spectrophotometer after 10 minutes at room temperature. The colour development is not stable. There is a 10-15% fading of colour intensity by 60 minutes. It is advisable therefore to only assay as many as samples at one time that can be comfortably read within about 20 minutes.

(j) Total ureide contents of sample are determined from the curve prepared from allantoin standards (the 250 mole standard should give an optical density reading of between 1.0 and 1.4), and a correction factor used (i.e. if 0.05mL samples were used, the factor is 1.0/0.05 = *20) to convert sample nmole determinations to nmole/mL.

2. Total amino acids ninhydrin method

[Reference: Yemm and Cocking, 1955. An adaptation of the method detailed below is described in Herridge, 1984.]

2.1 Reagents

A. 0.2 citrate buffer

Citric acid	21 g
NaOH (analytical grade)	8 g
Distilled water	500 mL

Check pH and adjust if necessary to pH 5.0.

B. Ninhydrin reagent

0.01 M potassium cyanide (65 mg in 100 mL distilled water) (stable 3 months at 20 C, do not pipette by month)	10 mL
Methoxy-ethanol (analytical grade)	590 mL
ninhydrin	5 g

The ninhydrin reagent should be prepared at least 24 hour before use. The reagent is very light-sensitive and should be stored away from light in a brown-glass bottle. Stable for only 2 weeks at room temperature. Stability can prolonged by storage at 4°C.

C. Amino acid standards

Prepare freshly for each day's analyses a 2.5 umole/mL, 50:50 asparagine:glutamine (most common amino compounds in xylem sap) stock solution.

Asparagine (store with desiccant)	16.5 mg

Glutamine (store with desiccant) 18.2 mg

Distilled water 100 mL

Dilute stock—

0.1 mL to 10 mL with distilled water (25 nmole/mL) 0.5 mL sample for analysis = 12.5 nmole

0.2 mL to 10 mL with distilled water (50 nmole/mL) 0.5 mL sample for analysis = 25 nmole

0.4 mL to 10 mL with distilled water (100 nmole/mL) 0.5 mL sample for analysis = 50 nmole

0.8 mL to 10 mL with distilled water (200 nmole/mL) 0.5 mL sample for analysis = 100 nmole

1.2 mL to 10 mL with distilled water (300 nmole/mL) 0.5 mL sample for analysis = 150 nmole

Note: Always include 0.5 mL distilled water blanks with standards during analysis.

2.2 Procedure

(a) Place 0.5 mL sample (20-50 uL xylem sap +450-480 uL distilled water) in each test-tube. (For preparation of amino standard curve use 0.5 mL of each amino acid standard and duplicate 0.5 mL water blanks

(b) Add 1.0 mL 0.2 M citrate buffer (pH 5.0), and

(c) add 1.2 mL ninhydrin reagent.

(d) Mix well and place in a boiling water-bath for 10-15 minutes.

(e) Remove from water-bath and cool to room temperature.

(f) Read optical density (absorbance) at 570nm on a spectrophotometer.

(g) Total amino acid content of samples is determined from a curve prepared from amino acid standards (the 250 nmole standard should give an optical density reading around 1.2), and a correction factor used to convert sample nmole determinations of nmole/mL.

3. Salicylic acid method for determination

[Reference: Cataldo et al, 1975. Suitable for all legume xylem sap samples tested to date except pigeonpea, with requires a metal reduction procedure such as described by Herridge (1984) for a accurate nitrate determinations.]

3.1 Reagents

A. Salicylic acid (5% w/v)

Salicylic acid	5 g
Concentrated sulphuric acid	100 mL

It is best if the salicylic acid reagent is prepared a few days before use. Once prepared it should be stable for several weeks.

B. 2 M NaOH

NaOH (analytical grade)	40 g
Distilled water	500 mL

3. Nitrate standards

Prepare a 25 umole/mL potassium nitrate stock solution

KNO3	0.253 g
Distilled water	100 mL

(sodium nitrate may be used if KNO3 not available)

Dilute stock—

0.5 mL to 10 mL with distilled water (1.25 umole/mL), 0.05 mL sample for analysis = 0.06 umole

1 mL to 10 mL with distilled water (2.5 umole/mL), 0.05 mL sample for analysis = 0.125 umole

1.5 mL to 10 mL with distilled water (3.75 umole/mL), 0.05 mL sample for analysis = 0.19 umole

2 mL to 10 mL with distilled water (5 umole/mL), 0.05 mL sample for analysis = 0.25 umole

2.5 mL to 10 mL with distilled water (6.25 umole/mL), 0.05 mL sample for analysis = 0.31 umole

Note: Always include 0.05 mL distilled water blanks with standards during analysis.

3.2 Procedure

(a) Place 0.05 mL sample of xylem exudate or hot water tissue extract in each test-tube. (For preparation of nitrate standard curve use 0.05 mL of each nitrate standard plus duplicate 0.05 mL water blanks.)

(b) Add 0.2 mL 5% salicylic acid and mix.

(c) Stand at room temperature for 20 minutes, then add 4.75 mL 2 M NaOH (to raise pH>12).

(d) Cool to room temperature and read optical density (absorbance) at 410 nm on a spectrophotometer.

(e) Nitrate contents of samples are determined from the curve prepared from nitrate standards (the 1 umole standard should give an optical density reading of around 1.2), and a correction factor used (1.0/0.05 = * 20) to convert sample umole determinations to umole/mL.

BIOGRAPHICAL SKETCH

The author, comes from Zhejiang province in the south-eastern China, was born on March 4, 1955. He completed his primary, secondary and high school education in the local area of Shangyi county, Zhejiang province in 1972, then worked as a farmer for three years and middle school teacher for two and half years in his hometown. After graduating with the degree of Bachelor of Science in the field of agronomy from Zhejiang Agriculture University in 1982, he became a staff member in the Institute of Agricultural Resource Utilization and Zoning, Zhejiang Academy of Agricultural Sciences.

In 1988, he was awarded a scholarship granted by the IDRC under the Asian Farming System Net Work through Chinese Academy of Agricultural Sciences to pursue his Master of Science in Agricultural Systems at Chiang Mai University, Thailand.

Wang Guofa

September, 1990