REFERENCES

Allen, O.N. and E.K. Allen. 1981. Leguminosae: A Source Book of Characteristics, Uses, and Nodulation. Univ. of Wisconsin Press, Madison.

Allos, H.F. and W.V. Bartholomew. 1959. Replacement of symbiotic fixation by available nitrogen. Soil Sci. 87: 61-66.

Andrews, C.S., A.D. Johnson and R.L. Sandland. 1973. Effect of aluminium on the growth and chemical composition of some tropical and temperate pasture legumes. Aust. J. Agric. Res. 24: 325-339.

Antoun H, C., J. Beauchamp, N. Goussard, R. Chabot and R. Lalande. 1998. Potential of *Rhizobium* and *Bradyrhizobium* species as plant growth promoting rhizobacteria on non-legumes: effect on radishes (*Raphanus sativus* L.) Plant Soil 204:57-67.

Azevedo, J. L., W.Jr. Maccheroni, J.O. Pereira and W.L. Araujo. 2000. Endophytic microorganisms: a review on insect control and recent advances on tropical plants. EJB Electronic J. Biotechnol. 3(1): 40-65.

Bashan, Y. 1998. Inoculants of plant-promoting bacteria for use in agriculture. Biotechnol. Adv. 16: 729-770.

Berkum, P. and B.D. Eardly. 1998. Molecular evolutionary systematics of the Rhizobiaceae. *In* The Rhizobiaceae: Molecular Biology of Model Plant-Associated Bacteria. (Spaink, H.P. *et al.* eds.), Kluwer Academic Publishers, Dordrecht. pp.1-24.

Bernstein, L. and G. Ogata. 1966. Effect of salinity on nodulation, nitrogen fixation and growth of soybean and alfafa. Agron. J. 68: 201-203.

Bezdicek, D. F., D. W. Evans, B. Abede and R. E. Witters. 1978. Evaluation of Peat and granular inoculum for soybean yield and N fixation under irrigation. Agron. J. 70: 865-868.

Bien, P.V. H.H. Tien, P.N. Quy, M.T. Tam, and B.V. Nu. 1996. The Soybean Agriculture Public House. Hanoi. 78pp. (in Vietnameses)

Bottomley, P. J. and D. D. Myrold. 2007. Biological N inputs. *In* Paul. (Ed.) Soil Microbiology, Ecology, and Biochemistry. Oxford Academic Press in an imprint of Elsevier. pp. 377.

Brady, D.J., C.H. Hecht-Buchholz, C.D. Asher, and D.G. Edwards. 1990. Effects of low activities of aluminium on soybean (*Glycine max*) 1. Early growth and nodulation. *In* van Beusichem, M.L. (Ed.) Plant Nutrition-Physiology and Applications. Kluwer Academic Publishiers, Dordrecht. pp. 329-334.

Brockwell, J., P.J. Botomley and J.E. Thies. 1995. Manipulation of rhizobial microflora for improving legume productivity and soil fertility: a critical assessment. Plant Soil 174: 143-180.

Bremer, E., C. van Kessel, L. Nelson, R.J. Rennil and D.A. Rennie. 1990. Selection of *Rhizobium leguminosarum* strains for lentil under growth room and field conditions. Plant Soil 121:47-56.

Caldwell, B.E. and G. Vest. 1968. Nodulation interactions between soybean genotypes and serogroups of *Rhizobium japnicum* strains on soybean yields. Crop Sci. 10: 19-21.

Caldwell, B.E. and G. Vest. 1970. Effect on *Bradyrhizobium japonium* strain on soybean yields. Crop Sci. 10: 19-21.

Campo, R.J. 1995. Residual Effects of Aluminium on *Bradyrhizobium japonicum* in Defined Medium and Soil Solution. Ph.D. Thesis. The University of Reading, Reading.

Catado D.A., M. Haroon, L.E Schrader and V.L. Youngs. 1975. Rapid colorimetric determination of nitrate in plant tissues by nitration of salicylic acid. Comm. Soil Sci. Plant Anal. 6: 71-80.

Cattelant, A.J., and M. Hungria, 1994. Nitrogen nutrition and inoculation. *In* Tropical Soybean: Improvement and Production. FAO. Rome. pp. 201-212.

Cooper, J.E., M. Wood and A.J. Holding. 1983. The influence of soil acidity factors on rhizobia. *In* Jones, D. G. and D. R. Davies (Eds.) Temperate Legumes: Physiology, Genetics and Nodulation. Pittman, London. pp. 319-335.

Coventry, D.R. and J. Evans. 1989. Symbiotic nitrogen fixation and soil acidity. *In* Robison, A.D. (Ed.) Soil Acidity and Plant Growth. Academic Press, Sydney. pp. 103-137.

Date, R. 1996. Selection of strains for inoculant production. *In* Balatti, A. P. and J. R. J. Freire. (Eds.) Legumes Inoculants: Selection and Characterization of Strains, Production, Use and Management. pp. 184.

Dara, P. and B. Martin, U. Sopheap, E. Natalie and B. McCorkell. 2006. Rhizobial inculation versus nitrogen fertilizer for mungbean, peanut and soybean in rainfed upland areas of Cambodia. Document from Cambodian Agricultural Research and Development Instititute. pp. 12.

Dart, P.J. 1974. Development of root-nodule symbioses. *In* Quispel. A. (Ed.) The Biology of Nitrogen Fixation. American Elsevier Publishing Company, New York. pp. 381-429.

Denarie, J., F. Debelle and C. Rosenberg. 1992. Signalling and host range variation in nodulation. Annual Review of Microbiology 46:453-464.

Diatloff, A. and S. Langford. 1975. Effective natural nodulation of peanuts in Queensland. Queensl. J. Agric. Anim. Sci. 32: 95-100.

Dilworth, M.J., J.G. Howieson, W.G. Reeve, R.T. Tiwari and A.R. Glenn. 2001. Acid tolerance in legume root nodule bacteria and selecting for it. Aust. J. Expt. Agric. 41: 446-453.

Eaglasham, A.R., J.S. Hassouna and R. Seegers. 1983. Fertilizer-N effect on N_2 fixation by cowpea and soybeans. Agron. J. 75: 61-66.

Erdman, L.W. and U.M Means. 1952. Use of total yield for predicting nitrogen content of inoculated legumes grown in sand cultures. Soil Sci. 73:231-235.

Evans, H.J. and L.E. Barbar. 1977. Biological nitrogen fixation for food and fiber production. Science 197: 332-339.

Ferreira, E.M and J.F. Margues. 1992. Select of portuguese *Rhizobium leguminosarum bv. trifolii* strains for production of legume inoculates. Plant and Soil 147: 151-158.

FAO. 1984. Legume Inoculants and Their Use. Food and Agriculture Organization of the United Nation. Rome. pp. 63.

FAO. 1998. Crop production. FAOTAT Database 1995, FAO Rome.

Giller, K.E. and K.J. Wison. 1993. Nitrogen Fixation in Tropical Cropping Systems. CAB International, Wallingford, pp. 313.

Gomez, K.A and A.A Gomez. 1984. Statistical Producers for Agricultural Research. 2nd Ed, John Wiley and Sons Inc., New York.

Graham, P.H., K.J. Draeger, M.L. Ferrey, M.G. Conroy, B.E. Hammer, E. Martinez, S.R. Aarons and C. Quinto. 1994. Acid pH tolerance in strains of *Rhizobium* and *Bradyrhizobium,* and initial studies on the basis for acid tolerance of *Rhizobium tropici* UMR 1899. Can. J. Microbiol. 40: 198-207.

Hadri, A_Z-E., H.P. Spaink, T. Bisseling and N.J Brewin. 1998. Diversity of root nodule and rhizobial infection process. *In* Spaink, H. P., A. Konlorosi and P. J. J. Hooykaas. (Eds). The Rhizobiaceae: Molecular Biology of Model Plant. Associated Bacteria. Kluwer Academic Publishers. London. pp. 347-360.

Halliday, J. 1984. Principles of *Rhizobium* strain selection. *In* Alexander, M. (Ed.), Biological nitrogen Fixation: Ecology, Technology and Physiology. Plenum Press, New York. pp. 155-171.

Ham, G.E., L.R. Frederic and I.C. Anderson. 1971. Serogroups of *Rhizobium japonicum* in soybean nodules. Agron. J. 63: 69-72.

Herridge, D.F. 1984. Effects of nitrate and plant development on the abundance of nitrogenous solutes in root-bleeding and vacuum extracted exudates of soybean. Crop Sci. 24: 173-179.

Herridge, D.F and M.B. Peoples. 2002. Timing of xylem sampling for ureide analysis of nitrogen fixation. Plant and Soil 238: 57-67.

Herridge, D.F 2002. Legume N and inoculants: global and Vietnamese perspectives. *In* Herridge. D. (Ed.) Inoculants and Nitrogen Fixation of Legumes in Vietnam.

Hinson, K., and H.H. Hartwig, 1982. Soybean Production in The Tropics. FAO. Rome. 158p.

Hobbs, R.L.A., J.D. Mahon. 1982. Effects of pea (*Pisum sativum*) genotypes and *Rhizobium legumninosarum* strains on N_2 (C_2H_2) fixation and growth. Can. J. Bot. 60: 2594-2600.

Howieson, J.G., G.W.O. Hara and S.J. Carr. 2000. Changing roles for legumes in Mediterranean agriculture: developments from an Australian perspective. Field Crops Research 65: 107-122.

Hungria, M., M.A.T. Vargas and R.S. Araujo. 1997. Fixacao biological do nitrogenio em feijoeiro. *In* Vargas, M.A.T., and M. Hungria (Eds.) Biologia dos Solos doc Cerrados. Brazil: EMBRAPA-CPAC, Planaltina. Brazil. pp. 189-295.

Hymowitz, T., R.J. Singh and K.P. Kollipara. 1998. The genomes of the *Glycine. In* Janick, J. (Ed.) Plant Breeding Reviews. 16: 289-311.

ITTA. 1985. Grain legume Improvement Program. Research highlights 1981-1984. Nigeria. pp. 80.

Jordan, D.C. 1982. Transfer of *Rhizobium japonicum* Buchanan 1980 to *Bradyrhizobium* gen. nov., a genus of slow-growing root-nodule bacteria from leguminous plants. Int. J. Syst. Bacteriol. 32: 136-139.

Jordan, D.C. 1984. *Rhizobiaceae.* CONN 1983. *In* Krieg, N.R. and J.G. Holt (Eds.), Bergey's Mannual of systematic Bacterology, Vol. I. Williams and Wilkins, Baltimore. pp. 234-244.

Kim, M.M., C.J. Asher, D.G. Edwards and R.A. Date. 1985. Aluminium toxicity: effects on growth and nodulation of subterranean clover. Proceedings of the 15 International Grasslands Congress, Kentucky. pp. 501-503.

Kuykendall, L.D., and D.F. Weber. 1978. Genetically marked *Rhizobium* identifiable as inoculum strain in the nodules of soybean plants grown in fields populated with *Rhizobium japonicum.* Appl. Environ. Microbiol. 36: 915-919.

Kuykendall, L.D., B. Saxena, T.E. Devine and S.E. Udell. 1992. Genetic diversity in *Bradyrhizobium japonicum* Jordan 1982 and proposal for *Bradyrhizobium elkanii* sp. nov. Can. J. Microbiol 38: 501-505.

Ladha, J.K., and M.B. Peoples, 1995. Management of Biological N_2 Fixation of the Development of More Productive and Sustainable Agricultural Systems. Kluwer Academic Publishers. pp. 125.

Libbenza, K.R. and R.J. Bogers. 1974. Root-nodule morphogenesis. *In* Quispel, A. (Ed.) The Biology of Nitrogen Fixation. American Elsevier Publishing, Inc., New York. pp. 430-472.

Lopez-Garcia, S.L., T.E.E. Vazouez, G. Favelukes and A.R. Lodeiro. 2001. Imporved soybean root association of N-starved *Bradyrhizobium japonicum.* J. Bacteriol. 183: 7241-7254.

Lowendorff, H.S. 1981. Factor affecting survival of *Rhizobium* in soil. Adv. Microbial Ecol. 4: 87-124.

MAFF. 2008-2009. Annual Report for Agriculture, Forestry and Fisheries. Ministry of Agriculture, Forestry and Fisheries. Phnom Penh, Cambodia. pp. 105.

MAFF. 2006-2010. Agricultural Sector Strategic Development Plant. Ministry of Agriculture, Forestry and Fisheries. Phnom Penh, Cambodia. pp. 49.

Matsukuma, S., T. Okuda, and J. Watanabe. 1994. Isolation of actinimycetes from pine litter layers. Actnomycetol. 8 (2): 57-65.

Moawad, A.M. 2000. Soil Biology. Institute of Agronomy in the tropics. University of Gottingen, Germany.

Mulonggoy, K., and M. Gueye. 1990. Biological N_2 fixation and Sustainability of Tropical Agriculture. Jones Wiley and Sons Inc., New York. 435p.

Munns, D.N. 1977. Mineral nutrition and the legume symbiosis. *In* Hardy, R.W.E and H. Gibson. (Eds.) A Treatise on Dinitrogen Fixation. Section IV. Agronomy and Ecology. John Wiley and Sons. New York. pp. 353-391.

Murphy, H.E., D.G. Edward and C.J. Asher. 1984. Effects of aluminum on nodulation and early growth of four tropical pasture legumes. Aust. J. Agric. Res. 35: 663-673.

Novozamsky, I., R. van Eck, J.Ch. van Schouwenburg and I. Walinga. 1974. Total nitrogen determination in plant material by means of the indophenol blue method. Neth.J.agric.Sci. 22:3-5.

Nguyen Xuan Cu. 1996. The performance of soybean on low phosphorous acid soil in the northern mountainous region of Vietnam. MSc Thesis. Chiang Mai University, Thailand. pp. 41-45.

Okazaki, T., K. Takahashi, M. kizuka and R. Enokita. 1995. Studies on acinomycetes isolated from plant leaves. Ann. Rep. Sanko Res. Lab. 47:97-106.

Paulitz, T.C and R.G. Linderman. 1991. Lack of antagonism between the biocontrol agent *Gliocladim virens** and vesicular arbuscular mycorrhizal fungal. New phytol. 117: 303-308.

Peoples, M.B., F.J. Bergersen, D.F. Herridge, M.N. Sudin, A.W. Faizah, K. Chong and M. Norhayati, 1988. Estimation of nitrogen fixation in legumes in the Tropics by xylem sap analysis. *In* Shanmsuddin, Z.H, W.M.W. Othman, M. Marziah and J. Sundram. (Eds.) Biotechnology of Nitrogen Fixation in the tropics. Serdang, Malaysia: Universiti Pertanian Malaysia, Serdang, pp. 117-126.

Peoples, M.B., A.W. Faizah, B. Rerkasem and D.F. Herridge. 1989. Methods for Evaluating Nitrogen Fixation by Nodulated Legumes in the Field. ACIAR Monograph No. 11, pp. 39-40.

Perret, X., C. Staechelin and W.J. Broughton. 2000. Molecular basis of symbiotic promiscuity. Microbiology and molecular biology reviews 64: 180-201.

Phillips, D.A., W.R. Streit, H. Volpin and C.M. Joseph. 1997. Plant regulation of root colonization by *Rhizobium meliloti. In* Legocki, A., H. Bothe, and A. Puhler. (Eds.) Biological Fixation of Nitrogen for Ecology and Sustainable Agriculture. Springer-Verlag, Berlin. pp. 133-136.

Polsnelli, M. and P.G. Mazza. 1984. Use of membrane filters for selective isolation of actinomycetes from soil. Microbiol. Lett. 22: 79-83.

Pueppke, S.G. 1986. Nodule distribution on legume roots: Specificity and response to the presence of soil. Soil Biol. and Biochem. 18:601-606.

Quispel, A. 1992. A search for signals in endophytic microorganisms. *In* Verma D.P.S. (Ed.) Molecular Signals in Plant-Microbe Communications. Boca Raton: CRC Press, Boca Raton. pp. 471-490.

Rennie, R.J. and S. Dubetz. 1984. Multistrain vs. single strain *Rhizobium japonicum* inoculants for early maturing soybeans cultivers: N_2 quantified by N^{15} isotopic dilution. Agron. J. 76: 498-502.

Rosas, J.C., J.A. Castro, E.A. Robleto, J. Handelsman. 1998. A method for screening *Phaseolus vulgaris* L. germplasm for preferential nodulation with a selected *Rhizobium etli* strain. Plant Soil 203:71-78.

Sadowsky, M.J. and P.H. Graham. 1998. Soil biology of the *Rhizobiaceae. In* Spaink, H.P., A. Kondorosi and P.J.J. Hooykaas. (Eds.) *The Rhizobiaceae:* Molecular Biology of Model Plant-Associated Bacteria. Kluwer Academic Publishers, Dordrecht. pp. 155-172.

Sardi, P., M. Saracchi, S. Quaroni, B. Peterolini, G.E. Borgonovi and S. Merli. 1992. Isolation of endophytic *Spreptomyces* strain from surface-sterile roots. Appl. Environ. Microbiol. 58(8): 2691-2693.

Sawada, H., L.D. Kuykendall and J.M. Young. 2003. Changing concepts in the systematics of bacterial nitrogen-fixing legume symbiosis. J. Gen. Appl. Microbiol 49: 155-179.

Schlaman, H.R.M., D.A.Phillips and E. Kondorosi. 1998. Genetic organization and transcriptional of rhizobial nodulation genes. *In* Spaink, H.P., A. Kondorosi and P.J.J. Hooykaas. (Eds.) *The Rhizobiaceae:* Molecular Biology of Model Plant-Associated Bacteria. Kluwer Academic Publishers, Dordrecht. pp. 361-386.

Senaratne, R., C. Amornpinol and G. Hardarson.1987. Effect of combined nitrogen on nitrogen fixation of soybean (*Glycine* max L. Merril) as affected by cultivar and rhizobial strain. Plant Soil 103:45-50.

Sessitsch, A., B. Reiter and G. Berg. 2004. Endophytic bacterial communities of field grown potato plants and their plant-growth-promoting and antagonistic abilities. Can. J. Microbiol. 50(4):239-249.

Shimizu, M., Y. Nakagawa, Y. Sato, T. Furumai, Y. Igarashi, H. Onaka, R. Yoshida and H. Kunoh. 2000. Studies on endophytic actinomycetes (I) *Streptomyces sp.* isolate from rhododendron and its antifungal activity. J. Gen. Plant Patho. 66: 360-366.

Sinha, S.K. 1977. Food Legumes Distribution Adaptability and Biology of Yield. FAO. Rome. pp. 230.

Somasegaran, P. and H.J. Hoben. 1994. Handbook for Rhizobia: Methods in Legume Rhizobium Technology. Springer-Verlag Inc., New York. Pp. 450.

Samac. D.A, A. M. Willert, M.J.Mcbride and L.L. Kinkel. 2003. Effect of antibiotic producing *Streptomyces* on nodulation and leaf spot in alfalfa. Applied soil ecology 22: 55-66.

Thapanapongworakul, P. 2003. Characterization of Endophytic Actinomycetes Capable of Controlling Sweet Pea Root Rot and Effects on Root Nodule Bacteria. MS. Thesis, Graduate School, Chiang Mai University. pp. 109.

Tanner, J.W. and D.J. Hume. 1978. Management and production. *In*. Geoffreyy, N. (Ed.) Soybean Physiology, Agronomy and Utilization. Academic Press, New York. pp. 164-166.

Thompson J.A., A. Bhromsiri, A. Shutsrirung, and S. Nillakan. 1991. Native root-nodule bacteria of traditional soybean growing areas of Northern Thailand. Plant Soil 135: 53-65.

Tokala, R.K., J.L. Strap, C.M. Jung, D.F. Crawford, M.H. Salove, L.A. Deobalb, J.F. Bailey and M.J. Morra. 2002. Novel plant-microbe rhizosphere interaction involving *Streptomyces lydicus* Wyce108 and the pea plant (*Pisium sativum*). Appl. Environ. Microbiol 68 (5): 2161-2171.

Vander Merwe, S.R., B.W. Strijdom, and C.J. Uys. 1974. Groundnut response to seed inoculation under extensive agricultural practices in South African soils. *Phytophylactic* 6: 295-302.

Van Kammen, A. 1995. The molecular development of nitrogen fixing root nodules. *In* Tikhonovich I.A., N.A. Provorov, V.I. Romanov and W.E. Newton. (Eds.) Nitrogen Fixation: Fundamentals and Applications. Kluwer Academic Publishers. Dordrecht. pp. 9-14.

Vargas, A.A.T. and P.H. Graham. 1988. *Phaseolus vulgaris and Rhizobium* strain variation in acid-pH tolerance and nodulation under acidic conditions. Field Crops Res. 19: 91-101.

Vincent, J.M. 1976. A Manual for Practical Study of Root Nodule Bacteria. Blackwell Scientific Publications, Oxford. pp.164.

Young E.G and C.E. Conway. 1942. On the estimation of allantoin by the Rimini-Schryver reaction. J. Biol. Chem. 142:836-853.

Wadisirisuk, P. and R.W. Weaver. 1985. Importance of bacteroid number in nodules and effective nodule mass to dinitrogen fixation by cowpea. Plant and Soil 87:223-231.

Wallinga, I., W. Van Vark, V.J.G. Houba and J.J. Van der Lee. 1989. Soil and Plant Analysis. An series of Syllabi. Part 7 Plant Analysis Procedures. Wageningen Agricultural Univ., Dept. of Soil Science and Plant Nutrition. Wageningen.

Weaver, R.W. and L.R. Frederick. 1974. Effects of inoculum rate on competitive nodulation of *Glycine max* L. Merril. II. Field studies. Agron. J. 66.233-236.

Weaver, R.W. and P.H. Graham. 1994. Legume nodule Symbionts. *In.* Weaver, R.W., S. Angle, P. Bottomley, D. Bezdicele, S. Smith, A. Tabatabi and A. Wollum, (Eds) Methods of Soil Analysis Part 2 Microbiological and Biochemical Properties. Soil Sci. Soc. Amer. Inc. pp. 199-218.

Wilson, P.W., and W.W. Umbried. 1967. Fixation and transfer of nitrogen in the soybean. Zenthl. Bact. Etc. 2 Abt. 96:402-411.

Xu, L.M., C. Ge, Z. Cui, J. Li and H. Fan. 1995. *Bradyrhizobium liaoningense* sp. nov., isolated from the root nodules of soybeans. Int. J. Syst. Bacterial. 45: 706-711.

Yuan, W.M and D.L. Crawford. 1995. Characterization of *Streptomyces lydicus* WYEC108 as a potential biological agent against fungal root and seed rots. Appl. Environ. Micobiol. 61(8):3119-3128.

Zary K.W., J.C. Miller, Jr.R.W. Weaver, and L.W. Barnes. 1978. Intraspecific variability for nitrogen fixation in southern pea (*Vigna unguiculata L.* Walp). J. Amer. Soc. Hort. Sci. 103(6):806-808.

APPENDIX

APPENDIX A

1. Yeast Mannitol Broth (YMB); YMA*

Mannitol	10 g
K_2HPO_4	0.5 g
$MgSO_4.7H_2O$	0.2 g
NaCl	0.1 g
Yeast extract	0.5 g
Distilled water	1.0 liter

Adjust to pH 6.8. Autoclave at 121°C and 15 lbs. For all media every time in autoclave according to volume:

Up to 500ml	20 minutes
1000ml	30 minutes
2000-4000ml	40 minutes
5000-8000ml	60 minutes

For Yeast Mannitol Agar (YMA)*

Yeast mannitol broth	1.0 liter
Agar	15 g
Congo red1%	2.5 ml

* Taken from Somasegaran and Hoben (1994)

2. Inhibitory Model Agar-2 (IMA-2)*

Glucose	5 g

Soluble starch	5 g
Beef extract	1 g
Yeast extract	1 g
Nz-case	2 g
NaCl	2 g
$ClCO_3$	1 g
Agar	20 g
Distilled water	1.0 liter

* Taken from Shimizu *et al.*, 2000

3. N-free nutrient solution*

stock solutions	element	M	form	MW	g/l	M
1	Ca	1000	$CaCl_2.2H_2O$	147.03	294.1	2.0
2	P	500	KH_2PO_4	136.09	136.1	1.0
3	Fe	10	Fe-citrate	355.04	6.7	0.02
	Mg	250	$MgSO_4.7H_2O$	246.5	123.3	0.5
	K	250	K_2SO_4	174.06	87.0	0.5
	Mn	1	$MnSO_4.H_2O$	169.02	0.338	0.002
4	B	2	H_3BO_3	61.48	0.247	0.004
	Zn	0.5	$ZnSO_4.7H_2O$	287.56	0.288	0.001
	Cu	0.2	$CuSO_4.5H_2O$	249.69	0.100	0.0004
	Co	0.1	$CoSO_4.7H_2O$	281.12	0.056	0.0002
	Mo	0.1	$Na_2MoO_2.2H_2O$	241.98	0.048	0.0002

*Broughton and Dillworth (1970) cited by Somasegaran and Hoben (1994)

For each 10 liters of full strength culture solution, take 0.5 ml each of solution 1 to 4, then add to 5.0 liters of water, then dilute to 10 liters. Use 1N NaOH to adjust the pH to 6.6-6.8.

For plus N control treatments, KNO_3 (0.05%) is added given an N concentration of 70ppm.

4. Soil Testing Kit (Central laboratory, Faculty of Agriculture Chiang Mai University)

Soil sample preparation

- Collect the same amount of soil from each sport. Mixed well with soils from the same sampling area.
- Air dry the sample. Do not dry the sample under the sun or in the oven in order to prevent the change of plant nutrient content.
- Grid and sieve the soil.

4.1 Soil pH testing procedure

- Fill up half of 2 wells in the pH testing tray with a soil sample
- Add pH indicator No. 2 into the soil in the first well until the soil is saturated and then add 2 more drops.
- Mixed well the soil and indicator by using wooden stick. Leave for a while until the soil is settle down.
- Read the pH value from color chart having the same color as the indicator in the well.
- If the color of indicator in the well is yellow checking pH of the soil in second well by using pH indicator No.1.

- If the color of indicator in the well is purple checking pH of the soil in second well by using pH indicator No.3.
- Read the pH value from color chart having the same color as the indicator in the well.

Criteria for soil pH measurement

pH	Level of soil reaction	Effects on plants
4.5 and lower	Extremely acidic	Too acid for most plants
4.6 – 5.2	Very acidic	Too acid for most plants
5.3 – 6.0	Moderately acidic	Too acid for some plants
6.1 – 6.9	Slightly acidic	Suitable for most plants
7.0	Neutral	Suitable for most plants
7.1 – 7.5	Slightly basidic	Suitable for most plants
7.6 – 8.2	Moderately basidic	Too basidic for some plants
8.3 – 9.0	Very basidic	Too basidic for many plants
9.1 and higher	Extremely basidic	Too basidic for most plants

4.2 Measurement of Lime Requirement (LR)

4.2.1 Lime requirement testing kit

- Buffer solution
- Plastic tube with cap
- Plastic spoon and wooden stick
- Plastic droppers 3ml. and 1ml.
- pH indicator No. 3.
- Lime requirement testing color chart

4.2.2 Testing of lime requirement of soil

- Soil 3 spoons + 3 ml of buffer solution
- Leave for 30 minutes
- Shaking every 10 minutes (2 times)
- Take the clear supernatant by using 1ml dropper
- Add 4 drops into the well in the tray
- Soil solution (yellow)
- Add 1 drop of pH indicator No.3.
- The color of soil solution change to green
- Read the figure from the LR testing color chart and estimate the amount of lime needed from the table of the LR color chart.

Lime Requirement Estimation

pH of buffer	amount of lime need (kg/ha)		
	$CaCO_3$	dolomite lime stone	burned lime
6.8	1,057	969	781
6.6	2,112	1,944	1,562
6.4	3,169	2,912	2,344
6.2	4,356	4,006	3,225
6.0	5,412	4,981	4,006
5.8	6,731	6,194	4,981
5.6	8,319	7,650	6,156
5.4	10,425	9,594	7,719
5.2	13,462	12,388	9,962
5.0	17,294	15,906	12,794

Note: use only one kind of lime

4.3 Testing of available P content of the soil

Available P testing kit

4.3.1 Extraction of available P from the soil

- P1 solution
- Plastic spoon with wooden stick
- Paper for soil sample
- Plastic bottle for soil extraction 1 bottle/sample
- Plastic bottle for filtration 1 bottle/sample
- Plastic funnel for and filter paper 1 set/sample
- 10 ml. plastic syringe 1 unit

4.3.2 Kits for color development

- A set of standard P solution (low, high, very high)
- Color development powder 1 tube (should be used within 24 hours after mixing with P2 solution)
- P2 solution 1 bottle
- P3 solution 1 bottle
- Glass test tube 3 tubes for standard P
- Glass test tube for soil sample 1 tube/sample
- 10 ml. plastic syringe
- 3 ml. plastic dropper 1 unit
- 1 ml. plastic dropper

4.3.3 Measurement of available P testing

- Soil sample 1 spoon
- 10 ml. P1 solution

- Shake 1 minute

- Filter

- Add P2 solution 10 ml. in color development powder

4.3.4 Color development

- Add 1 ml. from each of standard P solution into each of class test tube by stating with the lowest concentration

- Add soil extracting solution into one glass test tube

- Add 1ml. of color developing solution per tube

- Add 3ml. of P3 solution

- Shake slightly and leave for 15 minutes

- Compare the color of soil sample with the color of a set of standard P

Criteria of available P measurement

color comparison with STD P	level of available P	content of available P in soil (mg P/kg soil)
P sample < P std 1	Low	< 10
P std 1 < P sample < P std 2	Moderate	10 – 40
P std 2 < P sample < P std 3	High	> 40
P std 3 < P sample	Very High	> 100

4.4 Method for testing of exchangeable K in the soil

Exchangeable K testing kit

4.4.1 Soil extraction

- Plastic spoon with wooden stick

- Paper for soil sample
- Plastic bottle for soil extraction 1 bottle/sample
- Plastic bottle for filtration 1 bottle/sample
- K1 solution one bottle
- Funnel for and filter paper 1 set/sample
- 12 ml. syringe 1 unit

4.4.2 K testing by turbidity

- A set of standard K solution (low, high, very high)
- K2 solution 1 bottle (should kept in refrigerator)
- K3 solution 1 bottle
- Turbidity testing chart

4.4.3 Method of exchangeable K testing

- Soil sample 3 spoon
- 12 ml. K1 solution
- Shake 15 minutes
- Filter
- Add 1 ml. from each of standard K solution into each of glass test tube by stating with the lowest concentration
- Add soil extracting solution into one glass test tube
- Add K2 solution into each tube (10-15 drops)
- Add 1ml. of K3 solution
- Shake slightly and leave for 3 minutes

- Compare the turbidity of color extract solution with those color of a set of standard K by placing the turbidity testing chart at the back of the tube

Criteria of exchangeable K measurement

turbidity comparison with STD K	**level of exchangeable K**	**content of exchangeable P in soil (mg K/kg soil)**
K sample < K std 1	Low	< 60
K std 1 < K sample < K std 2	Moderate	60 – 100
K std 2 < K sample < K std 3	High	> 100
K std 3 < K sample	Very High	> 300

5. Measurement of Nitrogen Fixation by leguminous

5.1 Determination of Allantion

(Young and Conwey, 1942)

Reagents:

1- NaOH (0.5 M)

2- Phenylhydrazine hydrochloride (0.33%)

(to be made fresh on each day of analysis and store in a brown bottle)

3- Potassium ferricyanide (0.833%)

(to be made fresh on each day of analysis and store in a brown bottle)

4- HCl (0.65 M)

5- HCl (10 M) store at $0^{o}C$

6- Allantion standard (1.0 **µ** mole/ ml)

Dilute stock for peppering standard curve

(1) 10 *n* mole/ ml (2) 20 *n* mole/ ml

(3) 30 *n* mole/ ml (4) 40 *n* mole/ ml

(5) 50 *n* mole/ ml

Note: Always includes 2.5 ml distilled water blank with standard during analysis. A full set of standards should be run through (0-125 n mole; take 2.5 ml from each dilute stock) to check linearity of response.

Procedure:

1 Pipette 0.05-0.01 ml of sap sample into each test tube and dilute to 2.5 ml with distilled water.

2 Add 0.5 ml of 0.5 M NaOH

3 Mix and place tubes in boiling water bath for 10-15 min.

4 Remove tubes and allow to cool to room temperature, then add 0.5 ml of 0.65 M HCl and 0.5 mL of 0.33% Phenylhydrazine hydrochloride to each tube.

5 Place tubes in boiling water bath for 2-4 min.

6 Cool tubes immediately water bath for 15 min.

(The rapidity of cooling is an important factor in technique, rapid cooling increased the intensity of the final color and lower temperatures also increase the color intensity)

7 After remove tubes from ice bath, add 2 ml of 10 N HCl (chilled 0°C) then 0.5 mL of potassium ferricyanide. (mix immediately after each addition of potassium ferricyanide).

8 Stand at room temperature for 10 min then measure the absorbance at 525 mn.

The color is not stable, in 60 minutes there is 8-15% fading of color intensity, therefore the measurement of sample should be finished within 20 min)

5.2 Determination of Amino Acids with Ninhydrin

(Yemm and Cocking, 1955)[An adaptation of the method by Herridge, 1984]

Reagents:

1- Citrate buffer pH 5 (citric acid 16.8% (w/v), NaOH 6.4% (w/v).

2- Ninhydrin reagent (ninhydrin 0.96% (w/v), ascorbic acid 0.033% (w/v) in 2-methoxyethanol)

3- Ethanol 60% (w/v)

4- Asparagine standard (2.5 μ mole/ ml)

Dilute stock for peppering standard curve

(1) 50 *n* mole/ ml (2) 100 *n* mole/ ml

(3) 150 *n* mole/ ml (4) 200 *n* mole/ ml

(5) 250 *n* mole/ ml

Note: Always includes 0.5 ml distilled water blank with standard during analysis. A full set of standards should be run through (0-125 n mole; take 0.5 ml from each dilute stock) to check linearity of response.

Procedure:

1- Pipette 0.05 ml of sap sample into each tube and dilute to 0.5 ml with distilled water.

2- Add 0.5 ml of citrate buffer.

3- Add 1.2 ml of ninhydrin reagent and mix well.

4- Place tube sin boiling water bath for 25 min.

5- Remove from boiling water bath and cool to room temperature then add 3 ml of 60% ethanol.

6- Measure the absorbance 570 mn.

5.3 Determination of Nitrate

(Catadol et al., 1975)

Reagents:

1- 2 N NaOH

2- 5% (w/v) Salicylic acid in concentrated H_2SO_4 (SA- H_2SO_4).

(5 g of salicylic acid in 100 ml of con. H_2SO_4 make fresh at least once each week and store in brown bottle)

3- Nitrate standard (2.5 µ mole KNO_3/ ml)

Dilute stock for preparation of standard curve

(1) 2.50 µ mole KNO_3/ ml (2) 5.00 µ mole KNO_3/ ml

(3) 7.50 µ mole KNO_3/ ml (4) 10.00 µ mole KNO_3/ ml

(5) 12.50 µ mole KNO_3/ ml

Note: Always includes 0.5 ml distilled water blank with standard during analysis. A full set of standards should be run through (0-0.5 µ mole; take 0.5 ml from each dilute stock) to check linearity of response.

Procedure:

1- Pipette 0.05 ml of sap sample into test.

2- Add 0.2 ml of 5% SA- H_2SO_4 to sap sample and mix well.

3- Stand at room temperature for 20 min, then add 4.75 ml of 2 N NaOH into tubes slowly (to raise the pH about 12).

4- Cool to room temperature and measured the absorbance at 410 mn.

5.4 Relative Ureide Index

$$\text{Relative ureide index (\%)} = \frac{\text{UreideN}}{\text{TotalsapN}} \times 100$$

Since one ureide molecule contains 4 N–atoms, ureide is calculate as 4 X ureide molar concentration. Total sap N is estimated as 4 X ureide + amino acid + nitrate.

The relative ureide index can be calculated as:

$$\text{Relative ureide index (\%)} = \frac{4 \times \text{ureide}}{(4 \times \text{ureide} + \text{amino acid} + \text{nitrate})} \times 100$$

5.5 Plant sample digestion

(Novosamsky et al., 1974)

Reagents

1- Sulphuric acid, 96% (w/w), c (H_2SO_4) = 18 mol/l (d = 1.84g/ml).

2- Hydrogen peroxide 30% (w/w)

3- Salicylic acid, powder.

4- Digestion mixture. Put 18 ml water in a 250 ml Erlenmeyer flask. While cooling, add in small portions 100 ml of sulphuric acid (1) (CAUTION). Then dissolve 6 g of salicylic acid (3) with the aid of a magnetic stirrer.

Remark

3- Hydrogen peroxide must be of analytical quality; a lower grade may be stabilized with EDTA, phosphate, or other interfering compounds.

4- Since the dried sample still contains about 10% (w/w) water, the sulphuric

acid is somewhat diluted to prevent the temperature from raising too high during addition of the digestion mixture which otherwise would result in loss of nitrate.

Procedure

Weigh, to the nearest 0.001 g, approximately 0.3 g of the dried plant material sample in a metal weighing funnel and transfer the sample to a 50 ml volumetric flask. Take care that all the plant material comes below the neck of the flask. Add 3.3 ml of the digestion mixture (4) and swirl carefully until all the plant material is moistened. Allow to stand overnight. Prepare also two blank digestions.

Heat the flask on a hot plate at 180°C for about one hour. Remove the flask from the plate, let cool, and add 5 drops of hydrogen peroxide (2). Place the flask on the hot plate and increase the temperature to about 280°C. Heat 5-10 minutes until the water has evaporated (appearance of white vapours). Remove the flask from the plate, let cool, add 5 drops of hydrogen peroxide (2), and heat again for 5-10 minutes to appearance of white vapours. Repeat this treatment until the digest has turned colourless.

Remove the flask from the plate and cool to room temperature. Add about 10 ml water and mix; swirl until most of the precipitate has dissolved. Make up to the mark with water, mix well and filter over coarse filter paper.

Remark

5- After moistening the sample with the digestion mixture (4), at least 2 hours are needed to form the nitro-salicylic acid compounds. A longer period (overnight), however, will prevent foaming later on.

6- When heating at 180°C, the sample will turn black and foam may be formed. If this reaches the neck of the flask, 1 or 2 drops hydrogen peroxide (2) should be added.

7- A precipitate may be formed when cooling after completing the digestion. The addition of 10 ml water then produces enough heat to dissolve the precipitate rapidly.

8- The digest is filtered to remove any SiO_2 that will otherwise dissolve gradually and then interfere in the determinations.

9- Do not use the volumetric flasks for other purposes.

10- Near the end of the digestion, the liquid becomes yellow due to the last portions of organic matter, or to Fe. If the yellow colour persists after extra addition(s) of H_2O_2 , then the presence of Fe should be assumed and the digestion considered as completed.

5.6 Determination of nitrogen in digested plant samples by colorimetry

Field of application

This determination may be carried out on

digest 2.2 (H_2SO_4-salicylic acid-H_2O_2-Se)

digest 2.2 (H_2SO_4-salicylic acid-H_2O_2)

If for any reason, this determination cannot be carried out, then determination 3.23 may be done.

Apparatus

Colorimeter or spectrophotometer

Reagents

1- Sodium hydroxide solution, c (NaOH) = 10 mol/l. Dissolve 200g of sodium hydroxide, NaOH, in about 400 ml water; allow to cool and make up to 500 ml.

2- Salicylate solution. Dissolve 110 g of salicylic acid, $C_7H_6O_3$ in 150 ml of sodium hydroxide solution (1) and make up directly with water to 250 ml. Prepare just before use.

3- Buffer solution pH 12.3. Dissolve 26.70 g sodium hydroxide phosphate dehydrate, $Na_2HPO_4.2H_2O$ in some water in a 2 liters volumetric flask. Add 10 ml of the sodium hydroxide solution (1) and dilute to volume with water. Measure the pH and adjust if necessary.

4- EDTA solution. Dissolve 4 g of disodium ethylene diamine tetra acetate dehydrate, $Na_2EDTA.2H_2O$ in 100 ml water.

5- Hypochlorite solution. A stock solution, containing approximately 1 M sodium hypochlorite in 0.1 M NaOH, should be purchased commercially. Dilute 20 ml of this stock solution with water to 100 ml. Prepare fresh daily.

6- Nitroprusside solution. Dissolve 50 ml of sodium nitroprusside dehydrate, Na_2-$[Fe(CN)_5NO].2H_2O$, in 100 ml water. Prepare just before use.

7- Mixed reagent I. Mix 50 ml of the salicylate solution (2) with 100 ml of the nitroprusside solution (6) and 5 ml of the EDTA solution (4).

8- Mixed reagent II. Mix 200 ml of the buffer solution (3) with 50 ml of the hypochlorite solution (5).

9- Stock solution, N concentration 2500 mg/l. Dissolve 11. 793 g of ammonium sulphate, $(NH_4)_2SO_4$, in water in a 1000 ml volumetric flask and make up to volume with water.

Remark

4- The final solution of sodium hypochlorite should contain 0.7% ± 0.1% of active chlorine. This will be true for solutions purchased from a supplier of chemicals; if, however, the hypochlorite was bought as bleach in a supermarket, a check on its concentration is recommended.

5- The mixed stock solution of 4.5.4 can be used.

Standard series

Pipette into 100 ml volumetric flask, that contain already about 50 ml water, 4.5 ml of concentrated sulphuric acid (96%). Mix and let cool. Then add 0 - 1.00 - 2.00 - 3.00 - 4.00 - 5.00 - 6.00 ml of the stock solution (9) and dilute to volume with water.

Dilute this standard series 1 + 9 (v/v) with water; this should be done together with the diluting of the digests. The diluted standard series has N concentrations of 0 – 2.5 – 5 – 7.5 – 10 – 12.5 – 15 mg/l.

Procedure

Dilute all digests 1 + 9 (v/v) with water.

Pipette 0.20 ml of the diluted sample digest, the diluted blank digests and the diluted standard series into test tubes. Add 3.0 ml of mixed reagent I (7) and mix. Then add 5.0 ml of mixed reagent II (8) and mix. Allow to stand for at least 2 hours. Measure the absorbance in a 1-cm cuvette at a wavelength of 660 nm. Plot a calibration curve, and read the N concentrations.

Remark

5- In 2 hours the blue color reaches its maximum intensity; it is stable for at least 10 hours.

6- The digests obtained according to 2.2 or 2.3 are 0.7 – 0.9 M in H_2SO_4. Any further dilutions (if the N concentrations would be higher than the highest standard) should be made with zero standard solution.

7- The test tubes must be used exclusively for this N determination. They must first be cleaned by taking a blank determination; there after they should be cleaned only with water.

Calculation

The nitrogen content of the dried plant material, expressed in mmol/kg N, is calculated by

$$0.714 * (a-b) * \frac{V}{W}$$

in which

a : concentration of nitrogen in the diluted sample digest, in mg/l;

b : concentration of N in the diluted blank digest, in mg/l;

v : total volume of digest at the end of the digestion procedure, in ml;

w : weight of plant material sample, in g.

APPENDIC B

Appendix Table 1.

ANOVA table for nodule, shoot and total plant dry weight, %Ndfa and shoot N uptake of DT 84 soybean variety in control room

sov	df	MS			
		nodule dry weight	shoot dry weight	total dry weight	total plant N uptake
block	4	0.00193	0.47313	0.53102	5.209E - 04
tr.	15	0.00473 **	0.77281 **	0.81004 **	8.930E - 04 **
error	60	0.00051	0.12321	0.13216	1.279E - 04

Appendix Table 2.

ANOVA table for nodule, root and shoot dry weight of DT 84 soybean variety at V_6 and $R_{3.5}$ stage

sov	df	MS					
		V_6			$R_{3.5}$		
		nodule dry weight	root dry weight	shoot dry weight	nodule dry weight	root dry weight	shoot dry weight
block	4	0.991E - 05	0.00738	0.05089	0.00128	0.05430	0.21736
tr.	11	3.266E - 04 **	0.02022 **	0.18688 **	0.00231 ns	0.17023 **	1.03575 **
error	44	7.533E - 05	0.00373	0.03904	0.00149	0.03299	0.26265

Appendix Table 3.

ANOVA table for relative ureide index (%), %Ndfa, shoot N uptake and amount of fixed N of DT 84 soybean variety at $R_{3.5}$ stage

sov	df	MS			
		relative ureide index (%)	%Ndfa	shoot N uptake	Amount of fixed N
block	4	165.00	275.51	0.4685E - 04	0.00040
tr.	11	1215.67 **	2150.03 **	0.3114E - 04 ns	0.00130 **
error	44	100.00	173.11	1.616E - 04	0.00019

Appendix Table 4.

ANOVA table for shoot and root dry weight, no. of pods per plant and seed yield per plant of DT 84 soybean variety at harvest stage

sov	df	MS			
		shoot dry weight	root dry weight	pods per plant	seed yield
block	4	0.12394	0.05337	19.7508	3.25469
tr.	11	0.66782 **	0.13689 **	40.5366 **	5.44028 **
error	44	0.24142	0.03238	9.0361	0.95148

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