UNIVERSITI PUTRA MALAYSIA

EFFECTS OF SOIL MOISTURE AND A MIXED CULTURE OF PHOTOSYNTHETIC RHIZOBIA AND BRADYRHIZOBIA ON REDUCING SUGARS AND SYMBIOTIC GROWTH OF SOYBEAN

ZAINAH JALIL

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by

ZAINAH JALIL

Thesis Submitted in Fulfilment of the Requirements
for the Degree of Master of Agricultural Science
in the Faculty of Agriculture
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Especially dedicated to,

My beloved parents and family,

and also unforgetable friends

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Abstract of thesis submitted to the Senate of Universiti Putra Malaysia
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February 1998

Chairman: Associate Professor Dr. Zulkifli Hj. Shamsuddin

Faculty: Agriculture

The main objective of this study was to observe the effect of soil moisture and mixed culture of photosynthetic rhizobia and bradyrhizobia on reducing sugars and symbiotic growth of soybean.

Isolation of the native bradyrhizobia from Bungor, Kangkung, Katong, Lating, Lubok Kiat and Munchong series was conducted. One isolate from Lubok Kiat series was selected and labelled as UPMR48.

Two separate experiments were conducted using photosynthetic rhizobia strains. The first experiment was a single inoculation of eight strains of

photosynthetic rhizobia to soybean. Two strains (MKAa2 and MKAa3) were found capable of forming root nodules. In the second experiment, photosynthetic rhizobia strains (MKAa2 and BTAi1) were inoculated to soybean as a single or mixed culture along with bradyrhizobial strains (UPMR48, TAL102 and CB1809). The best mixed culture was MKAa2 and UPMR48 strains.

Nodulation and growth patterns of soybean experiment was conducted with plants harvested at different plant age, 28 days (D_{28}), D_{35}, D_{42} and D_{49}, after planting. Maximum nodulation was observed at D_{42} (early pod filling stage).

The Water Level experiment was conducted using peat and mineral soil with a control soil moisture maintained at field capacity and water levels maintained at 7.5 cm. 15.0 cm, 20.0 cm, 25.0 cm, 30.0 cm (peat) and 7.5 cm, 15.0 cm, 22.5 cm, 30.0 cm (Lubok Kiat series) from the soil surface. Plants were harvested at D_{32} and D_{42} for peat and Lubok Kiat series, respectively. Maximum plant growth and nodulation were observed in conditions with water levels at 25 cm (peat) and 30 cm (Lubok Kiat series) from the soil surface; the soil moisture was at or above the field capacity (pF 2.0-2.5). In peat, root nodules were abundant at nearest to the maintained water level for all treatments while for Lubok Kiat at the initial 0-7.5 cm depth of soil surface irrespectively of the water level treatments.

Mixed culture (MKAa2 and UPMR48) inoculation of soybean grown under water level maintained at 30 cm from the soil surface and at field capacity increased symbiotic growth of soybean compared to a single inoculation of the respective strains. Plants inoculated singly with UPMR48 strain grew best when water level was maintained at 30 cm but with MKAa2 strain, soil moisture at field capacity was prefered.

Generally this study showed that the water level maintained at 30 cm from the soil surface and field capacity condition along with a mixed culture inoculation of photosynthetic rhizobia and bradyrhizobia (MKAa2+UPMR48) produced maximum reducing sugar concentration in nodules and symbiotic growth of soybean.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk Ijazah Master Sains Pertanian

KESAN LEMBAPAN TANAH DAN INOKULASI CAMPURAN KULTURA DI ANTARA 'PHOTOSYNTHETIC RHIZOBIA' DAN BRADYRHIZOBIA KE ATAS GULA PENURUN DAN PERTUMBUHAN SIMBIOTIK KACANG SOYA

Oleh

ZAINAH BTE JALIL

Februari 1998

Pengerusi: **Profesor Madya Dr. Zulkifli Hj. Shamsuddin**

Fakulti: **Pertanian**

Objektif utama kajian ini adalah untuk mengkaji kesan lembapan tanah dan inokulasi campuran di antara 'photosynthetic rhizobia' dan bradyrhizobia ke atas gula penurun dan pertumbuhan simbiotik kacang soya.

Pengasingan 'native' bradyrhizobia dari siri Bungor, Kangkung, Katong, Lating, Lubok Kiat dan Munchong telah dijalankan. Satu 'native' bradyrhizobia dari siri tanah Lubok Kiat dapat diasingkan dan dilabel sebagai UPMR48.

Dua eksperimen yang berasingan menggunakan strain ‘photosynthetic rhizobia’ dan bradyrhizobia telah dijalankan. Eksperimen pertama adalah inokulasi tunggal lapan strain ‘photosynthetic rhizobia’ ke atas kacang soya. Dua strain (MKAa2 dan MKAa3) berjaya menghasilkan bintil pada akar. Eksperimen kedua adalah inokulasi strain ‘photosynthetic rhizobia’ (MKAa2 dan BTAi1) secara tunggal atau campuran dengan strain bradyrhizobia (UPMR48, TAL102 dan CB1809). Campuran inokulum yang terbaik adalah di antara strain MKAa2 dan UPMR48.

Kajian corak pembintilan dan pertumbuhan kacang soya dijalankan di mana hasil dituai pada peringkat umur yang berbeza, 28 hari (D_{28}), D_{35}, D_{42} dan D_{49}. Pembintilan yang maksima adalah pada D_{42} (peringkat awal pembentukan lenggai).

Eksperimen Paras Air telah dijalankan menggunakan tanah gambut dan mineral dengan kawalan lembapan tanah pada tahap muatan ladang serta pengekalan paras air pada 7.5 sm, 15.0 sm, 20.0 sm, 25.0 sm dan 30.0 sm (gambut) dan 7.5 sm, 15 sm, 22.5 sm dan 30.0 sm (siri Lubok Kiat) dari permukaan tanah. Pokok dituai pada D_{32} untuk tanah gambut dan D_{42} untuk siri Lubok Kiat. Pertumbuhan dan pembintilan maksima didapati pada paras air 25 sm (gambut) dan 30 sm (siri Lubok Kiat) dari permukaan tanah; keadaan lembapan tanah adalah pada atau lebih daripada tahap muatan ladang (pF 2.0-2.5). Bagi tanah gambut, bintil banyak terdapat pada kawasan yang berdekatan dengan paras air untuk semua rawatan, manakala bagi Lubok Kiat pada kedalaman 0-7.5 sm dari permukaan tanah di semua rawatan paras air.

Inokulasi campuran (MKAa2 dan UPMR48) ke atas kacang soya yang ditanam di pengekalan paras air pada 30 sm dari permukaan tanah dan lembapan pada tahap muatan ladang meningkatkan pertumbuhan simbiotik kacang soya berbanding dengan inokulasi tunggal kedua-dua strain. Pokok yang diinokulasi secara tunggal dengan UPMR48 mempunyai pertumbuhan yang lebih baik pada paras air yang kekal pada 30 sm tetapi bagi strain MKAa2, adalah lebih baik pada lembapan tanah di tahap muatan ladang.

Keseluruhannya, kajian menunjukkan pengekalan paras air pada 30 sm dari permukaan tanah dan tahap muatan ladang serta inokulasi campuran 'photosynthetic rhizobia' dan bradyrhizobia (MKAa2+UPMR48) menghasilkan kandungan gula penurun dalam bintil dan pertumbuhan simbiotik yang maksimum.

CHAPTER 1

INTRODUCTION

Soybean has been established as an important source of plant protein for human consumption and animal utilization. In 1996, Malaysia has to import about RM 290 million (Department of Statistic, Malaysia, 1996) of soybean to fulfill the increasing trend in consumption and utilization of soybean for food and animal feed. Soybean is not a major crop in Malaysia due to the low grain yield, 1.0 to 3.0 tonnes ha^{-1} (Leong, 1989), its specific climatic requirement for a distinct dry period during pod filling and at harvest, and incidence of pests and diseases.

It is well known that soybean is the most important cultivated leguminous host plant for rhizobia which form root nodules, symbiotically fix atmospheric nitrogen and provide the nitrogen requirement of the host plant. In view of the escalating cost of chemical fertilizers, full exploitation of the symbiotic N_2 fixing process is important in soybean cultivation. However, there are many factors which could limit N_2 fixation including the effectiveness of the rhizobial inoculum and environmental conditions. The rhizobia to be used as inoculant should be carefully selected to ensure optimum performance in the nodulation of legume roots. A reliable rhizobia should be highly competitive, persistent and effective over a specified range of environmental conditions. Water availability (Pankhurst and Sprent, 1975; Rathore et al., 1981) and photosynthate supply from the host plant (Coker and Schubert, 1981; Gordon et al., 1985) are the most important environmental factors that could influence nodulation and subsequently N_2 fixation activity.

In the *Rhizobium*-legume symbiosis, soil moisture is critical for nodule formation and development (Minchin and Pate, 1975; Gallacher and Sprent, 1978), nodule function and consequently N_2 fixation (Albretch et al., 1981; Williams and De Mallorca, 1984). Several Australian researchers have shown that soybean could acclimatise to growth in a saturated soil (heavy clay) at high water levels of 3 and 15 cm from the soil surface (Troedson et al., 1984; Nathanson et al., 1984; Lawn and Byth, 1989). The system could increase water supply and water status in leaves which could consequently increase the rate of photosynthesis (Troedson et al., 1984). The rapid acclimation of the seedling was attributed to the profusion of root growth and nodulation in the saturated soil just above the water level which remained oxidized (Troedson et al., 1984). In Malaysia, this system has been used in the experiment conducted by MARDI (Malaysian Agricultural Research and Development Institute) to increase grain yield of several soybean varieties grown on padi soils (Leong and Ramli, 1992). However, there is insufficient evidence on the critical water levels and the optimum soil moisture required for maximum photosynthate production (especially reducing sugars), growth, nodulation and N_2 fixation in soybean.

In the *Rhizobium*-legume symbiosis, photosynthate is required by the bacteroid as an energy source (ATP) and a reductant in the reduction of organic nitrogen which are exported from nodules to leaves and pods, to generate carbon skeletons for amino acid or ureide synthesis (Rawsthorne et al., 1980; Coker and Schubert 1981). Many evidence have indicated that photosynthate from the host plant to the bacteroids is the major limiting factor for symbiotic N_2 fixation in leguminous plants such as soybean (Ryle et al., 1985) and cowpea (Parajasingham and Knievel, 1990). Generally, the amount of photosynthate transported down to the nodules decreases with age (Lie, 1981), particularly during the generative phase when the pods and seeds compete for the carbon and nitrogen with other plant organs.

In nature, photosynthetic bacteria could fix molecular nitrogen and assimilate carbon dioxide. Kobayashi_et al., 1981 showed that when *Rhizobium* was cultured together with a photosynthetic bacteria *(Rhodopseudomonas capsulata*), the *Rhizobium* could form nodules efficiently and the N_2 fixation activity increased. In the late 1980's, several researchers from Boyce Thompson Institute claimed to have isolated the first photosynthetic rhizobia (genus *Photorhizobium)* that could fix N_2 an be able to photosynthesize (Fleischman et al., 1991). The photosynthetic rhizobia was isolated from stem nodules of *Aeschynomene* plant grown on a submerged soil. Several studies have been done on the characteristics (Eaglesham et al., 1990) and the photosynthetic properties (Fleischman et al., 1991) of this rhizobia species. However the nodulating abilities of the photosynthetic rhizobia on other legumes such as soybean, inoculated singly or as a mixed culture with other genus of rhizobia such as *Bradyrhizobium*, are still unknown.

The objectives of this research are to observe the effect of soil moisture and mixed culture of photosynthetic rhizobia and rhizobia on reducing sugar, growth, nodulation and N_2 fixation of soybean.

CHAPTER II

LITERATURE REVIEW

Importance of Soil Moisture to Plant Growth

Plants need water for all metabolic processes and growth. Plaster (1992) reported that crop plants used about 227 to 318 kg of water to produce a single kg of growth such as being one of the largest constituent of plant cells (50% to 90%) , as a building block in the manufacture of carbohydrates, an agent to cool plant through transpiration or evaporation, a medium to make nutrients available to plants and also as a carrier of nutrient and carbohydrates throughout the plants.

Plants obtain rainfed and irrigated water through their extensive root systems. Generally, there are three soil moisture conditions, saturation (all the pore spaces between soil particles are filled with water), field capacity (water held by soil particles after surplus has been drained by gravity) and permanent wilting point (water so tightly held by soil particles that it cannot be absorbed by plants). At saturation, field capacity and permanent wilting point the soil retains moisture under a tension of pF 0, 2.5 and 4.2, respectively (FAO, 1971; Miller and Donahue, 1995).

Plant grows most rapidly at field capacity because there is enough soil air and sufficient water held loosely at high potential. However, there are crops which can grow best under saturated condition such as *Sesbania,* padi and recently soybean.

Influence of Soil Moisture to the Legume-Rhizobia Symbiosis

An adequate water supply is essential for normal plant growth, root development and many activities of plant processes such as the legume-rhizobia symbiosis. Soil moisture is critical for nodule formation (infection of rhizobia) and development (Minchin and Pate 1975; Gallacher and Sprent, 1978), nodule function and consequently nitrogen fixation (Albretch et al., 1981; Williams and De Mallorca, 1984).

Pankhurst and Sprent (1975) reported that 70% of N_2 fixing activity and 50% of the respiratory activity of detached soybean nodules were lost when the water potential of nodule was lowered from approximately -1×10^5 Pa (turgid nodule) to -9×10^5 Pa (moderately stressed nodules) and almost total lost of N_2 fixing activity and 80% loss of respiratory activity when the potential was -1×10^6 Pa.