Impacts of Water Deficit on Growth and Yield of Soybean under Different Soil Types

（異なる土壌条件下における節水管理がダイズの生育と収量に与える影響）

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SUMMARY

Soybean is economically important crop with widespread consumption, utilization in vegetable oil and veterinary industries. Soybean is a traditionally nonirrigated (rain fed) crop that occupies quite extensive areas in agro ecosystems. Drought is a worldwide problem, constraining global production and quality of crop seriously, and recent global climate change has made this situation more serious. The great challenge for the future will be the task of increasing food production with less water, particularly in countries with limited water and land resources. In the context of improving water use efficiency, there is a growing interest in "deficit irrigation". At present and more so in the future, irrigated agriculture will take place under water scarcity. To cope with scarce supplies, deficit irrigation, defined as the application of water below full crop-water requirements (evapotranspiration), is an important tool to achieve the goal of reducing irrigation water use. Another feature of the irrigation areas is soil types, which vary considerably in their infiltration, available water holding, and drainage properties. Different soil types are known to influence the total available water to plants and, therefore, the time when crop water stress develops during a period of drying. This effect is incorporated in deficit irrigation scheduling systems based on water balance estimates.

Based on the above description, a series of vinyl house with open surrounding sides experiments were conducted with soybean cultivar (*Glycine max L. Merrill*), located (35°27' N. and 136°44' E.) in the experimental farm of Gifu University, Japan, with the following objectives:

1. To evaluate the potentialities of the three soil types under various water deficit conditions to yield of soybean,
2. To elucidate the water stress effects on Soil Plant Analytical Development (SPAD) chlorophyll meter reading and its relationship to nitrogen status and grain yield of soybean under the three soil types,
3. To investigate the effects of water stress on root/shoot ratio, water use efficiency (*WUE*) and yield efficiency *(YE)* at different growth stages of soybean, and
4. To assess the effect of water stress on nodulation of uninoculated soybean and leaf N accumulation to grain yield at different growth stages of soybean.

To achieve the first and second objectives, an experiment was conducted in a vinyl house at Gifu University, Japan, from June to November 2007. The soil type was the first factor with three different soil types, comprising of clay loam, sandy clay loam, and sandy loam soils, classified as Inceptisol, Ultisol, and Andisol, respectively. Water deficit (*D*) was the second factor with four levels including D_1(0-25%), D_2(25-50%), D_3(50-75%) and D_4(75-100%) water deficits of total available water (*TAW*).

Achievement of the first objective:

The crop water requirement (*CWR*) of soybean in the three soil types significantly decreased with the increasing water deficit levels, and the highest was in Inceptisol, followed by Ultisol and then Andisol under all water deficit levels. Grain yield of soybean per unit area in Inceptisol was the highest, followed by Ultisol and then Andisol under all water deficit levels. The values of yield efficiency (*YE*), indicating the grain yield per unit *CWR*, was strongly influenced by water deficit level, and the maximum *YE* occurred at the water deficit level D_3 (50-75%) in all the three soil types. However, there were no significant differences at 5% level among the maximum values of *YE* in the three soil types. The lowest yield response factor (K_y), indicating the relative yield loss to relative water deficit, was seen in Inceptisol (K_y=0.42), followed by Ultisol (K_y=0.64) and then Andisol (K_y=0.87) under the water stress lower than 50-75% of *TAW*. These results indicate that deficit irrigation in Inceptisol contained the finest soil texture is the most effective for economic water usage among the three soil types under the water deficit lower than 50-75% of *TAW* (D_3).

Achievement of the second objective:

There were significant positive relationships between the soybean grain yield to the *ET* and *LAI*, and as well to the SCMR and N accumulated under the three soil types in response to different water deficit levels. These relationships indicate that the reduction in *ET* with the decrease of *LAI* by the water stress caused the decrease of both SCMR and nitrogen status and a subsequent decrease in soybean grain yield among the three soil types. Thus, the result suggests that seed formation stage is the best time for prediction of potential yielding ability of soybean grain through the measures of SPAD meter reading, because at this time leaf chlorophyll reaches its maximum. One could hypothesize, based on our results, that selection criteria of suitable soil types for large nitrogen assimilation could be an important soybean production goal under water stress conditions.

To achieve the third and fourth objectives, an experiment was conducted in a vinyl house at Gifu University, Japan, from June to November 2008. The experimental design was a randomized complete block of five treatments with nine replications. The treatment imposed was deficit irrigation with five levels including D_1 (0-20%), D_2 (20-40%), D_3 (40-60%), D_4 (60-80%), and D_5 (80-100%) water deficits of total available water *(TAW*). The three growth stages were flowering (49 DAS), seed growth (77 DAS), and maturity (140 DAS).

Achievement of the third objective:

Soybean seed yield (*Y*) significantly correlated to the crop water requirement (*CWR*) as well as to the leaf area index (*LAI*) and total dry biomass (*TDB*) in response to water deficit levels. These relationships indicated the water stress decreased *CWR* which in turn caused the decrease in *LAI* and *TDB* and a subsequent decrease in grain yield. However, *WUE* and *YE* values increased with increasing root/shoot ratio up to the D_4 treatment and thereafter, decreased up to the D_5 treatment in response to increasing water deficit levels. The study showed that the most effective economic water usage with the highest *YE* were at D_4 water deficit. It could produce 21% lower yield per plant, but could conserve 18% irrigated water to produce the same yield compared to the potential yield produced under full irrigation (D_1).

Achievement of the fourth objective:

The highest leaf N accumulation was in the D_2 treatment at the flowering and seed growth stage. The soybean grain yield had positive significant correlation ($p<0.01$) with leaf nitrogen at seed growth stage. Total nodule numbers at ≧4.75 mm diameter size had non-significant effect on leaf N accumulation, but had positive significant effect ($p<0.05$) on grain yield of soybean at seed growth and maturity stage. On the other hand, total nodule numbers at <4.75 mm size had positive significant effect ($p<0.01$) on leaf N accumulation and grain yield of soybean at seed growth stage. Total nodule fresh and dry weight at≧4.75 mm size had non-significant effect on leaf N accumulation and grain yield of soybean, but nodules at <4.75 mm size had a positive significant effect ($p<0.01$)

at seed growth stage. Individual nodule fresh and dry weight at ≥4.75 mm size showed negative significant correlation ($p<0.01$) with leaf N accumulation and grain yield, but nodules at < 4.75 mm size showed positive significant correlation ($p<0.01$) at seed growth stage. Our studies demonstrated that the water deficit level D_2 (20-40% of *TAW*) was the best for an efficient *Rhizobium*-host association and subsequent nodule development. Based on our results, it can be concluded that successful root infection of uninoculated soybean was more pronounced in < 4.75 mm diameter size class nodule than the larger ones (≥4.75 mm) under different water deficit levels.

PREFACE

This dissertation is a part of fulfillment of the degree of Doctor of Philosophy (PhD) under the supervision of Prof. Dr. Masateru Senge, of the United Graduate School of Agricultural Sciences, Gifu University, Japan. This thesis is a compilation of the results of vinyl house experiment, which was conducted at Gifu University, Japan, 2007 to 2009.

The research field is impacts of water deficit on soybean under different soil types. As water supplies declined and the cost of water increases, it is clear that producers are being driven toward deficit irrigation management. Japan has vast experience in irrigation and water management of fields for rain-fed crops grown. Bangladesh has many factors similar to those in Japan. Therefore, experience gained through the proposed research program, may be beneficially used to develop irrigation and water management system in Bangladesh.

Soybean is a traditionally nonirrigated (rain fed) crop that occupies quite extensive areas in agro ecosystems. The great challenge for the future will be the task of increasing food production with less water, particularly in countries with limited water and land resources. In the context of improving water use efficiency, there is a growing interest in "deficit irrigation". To cope with scarce supplies, deficit irrigation, defined as the application of water below full crop-water requirements (evapotranspiration), is an important tool to achieve the goal of reducing irrigation water use. Another feature of the irrigation areas is soil types, which vary considerably in their infiltration, available water holding, and drainage properties. Different soil types are known to influence the total available water to plants and, therefore, the time when crop water stress develops during a period of drying. This effect is incorporated in deficit irrigation scheduling systems based on water balance estimates.

This dissertation has been successfully completed following suggestion, invaluable cooperation and encouragement from many peoples, whom I cannot mention one by one. I would like to take opportunity to extend my appreciation to:

Words can hardly express my profound sense of gratitude and indebtedness to my supervisor Prof. Dr. Masateru Senge, Head of the Dept. Irrigation and Drainage, Faculty of applied Biological Science, Gifu University, who introduced me to this problem and

helped me to conduct this research work. I extend my hearty thanks and gratefulness to my reverent advisor Dr. Masateru Senge for his keen interest, enthaustic guidance, gratuitous teaching, valuable instruction, constructive criticism, constant encouragement and great inspiration throughout the research work, reading, commenting and tirelessly correcting the manuscript.

I like to take an opportunity of expressing my sincerest thanks and heartfelt gratitude to Prof. Dr. Kazuhiro Takamizawa, Dean of The United Graduate School of Agricultural Science, Gifu University, for a great opportunity to study in Japan.

Heartiest thanks to Associate Prof. Dr. Kengo Ito for constant help and cooperation, inspiration and encouragement for all the time.

I am grateful for the financial support of the Ph.D. program by the University of Dhaka, Bangladesh and supported in part by The United Graduate School of Agricultural Science, Gifu University, Japan.

Last but not the least, I am thankful to my friends and all students in laboratory who has received me and work together all the day.

The dissertation of the degree of Doctor of Philosophy (PhD) is “Dedicated to Almighty God, Parents and Family”.

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1. Introduction

Soybean is economically an important crop with widespread consumption and utilization in vegetable oil and veterinary industries. Soybean is a traditionally nonirrigated (rain fed) crop that occupies quite extensive areas in agro ecosystems. Soybean crop seldom attains its full yield potential because of limitations on physiological processes imposed by environmental stresses. Drought is one of the major abiotic constraints affecting soybean productivity and quality worldwide. Shortage of available water is one of the most significant environmental stresses that cause yield reductions in a wide range of crops including soybean (Frederick and Hesketh, 1994).

Drought is a worldwide problem, constraining global production and quality of crop seriously, and recent global climate change has made this situation more serious. The great challenge for the future will be the task of increasing food production with less water, particularly in countries with limited water and land resources. In the context of improving water use efficiency, there is a growing interest in "deficit irrigation". At present and more so in the future, irrigated agriculture will take place under water scarcity. To cope with scarce supplies, deficit irrigation, defined as the application of water below full crop-water requirements (evapotranspiration), is an important tool to achieve the goal of reducing irrigation water use. According to James (1988), full irrigation is economically justified when water is readily available and irrigation cost is low.

Water resources in many areas of the world are limited but their demand is increasing. Irrigation agriculture is under economic and political pressure to improve the efficiency with which water is used. Efficient use of water resources depends on reducing water losses, which can be minimized through use of new irrigation techniques such as irrigation programs with deficient evapotranspiration. Demand for evapotranspiration can be reduced either through agronomic measures or use of deficit irrigation programs. The main approach in deficit irrigation practice is to increase crop water use efficiency by partially supplying the irrigation requirement and allowing water stress to planned plant with the least impact on crop yield. Deficit irrigation management requires optimizing the degree of plant stress within the restriction of available water.

Another feature of the irrigation areas is soil types, which vary considerably in their infiltration, available water holding, and drainage properties. Different soil types are known to influence the total available water to plants and, therefore, the time when crop water stress develops during a period of drying. This effect is incorporated in irrigation scheduling systems based on water balance estimates. However, it is generally assumed that the rate of transpiration from a crop with full canopy development and adequate water is controlled only by atmospheric condition and by physical and physiological properties of the canopy with soil type having little or no effect. There are few experiments on evapotraspiration from crop species grown on different soil types. Also, the reports of soil type effects on total water use by a well-watered crop exist, but the cause of water deficit effects is not known.

Recent research indicates a close link among leaf chlorophyll concentration, leaf N content and crop yield, which makes sense because the majority of leaf N is contained in chlorophyll molecules (Cartelat *et al.*, 2005; Lopez-Bellido *et al.*, 2004). The proportion of leaf N allocated to the chloroplast amounts to approximately 75% (Huk *et al.* 1993). The soil plant analytical development (SPAD) chlorophyll meter reading (SCMR) enables users to quickly and easily measure leaf greenness (by measuring the leaf light-transmittance characteristics) which is affected by leaf chlorophyll content. The usefulness of SPAD chlorophyll meter readings for plant N assessment is based on the direct proportionality between leaf chlorophyll and leaf N content (Sheshshayee *et al.*, 2006). A number of factors, one being N status of the plant, affect chlorophyll content or leaf greenness (Richardson *et al.*, 2002). Plant water stress can affect the ability of the plant to produce chlorophyll, thus affecting leaf greenness (Sandoval-Vila *et al.*, 2002).

Improvements in yield efficiency of crops through water use efficiency are essential under the scenarios of water scarcity predicted by global climatic changes. Direct impacts of drought stress to the physiological development of soybean depend on its water use efficiency (*WU*E) (Earl, 2002). In agriculture management involving soybean as a crop, *WUE* is an important physiological characteristic related to the ability of plants to cope with water stress. According to Passioura (1997), grain yield (*Y*) is a function of the amount of water transpired, *WUE*, and harvest index. Soybean, as a C3 plant, is less efficient in water-use due to high evapotranspiration and low photosynthetic rates.

Numerous studies have shown that soil water stress has significant effects on plant growth. Far fewer studies have focused on the root:shoot ratio response of plants, especially soybean crops, to water stress. Water stress in soybean has been shown to reduce growth of above-ground organs, leaf photosynthesis and leaf transpiration. However, studies are not available on the adaptation of soybean crop to water stress and its effects on root:shoot ratio for water uptake and its relationship to water use efficiency (*WUE*) and yield efficiency (*YE*).

Nodulation and leaf nitrogen (N) accumulation in soybean are sensitive to water deficit conditions, and can have important significant effects on yield. There have been numerous studies on the relationship between soil moisture and activities of soil microorganisms as well as nodulation (Hill *et al.*, 2000). It is also known that moisture stress affects various physiological processes in plants (Gan *et al.*, 2008). A disturbed water metabolism of the macrosymbiont may cause an impairment of the soil-plant-water balance, which may lead to reduce N_2 fixation and uptake (Upreti and Murti, 1999). The soil moisture that is adequate for seed germination is also adequate for bacterial activity and nodules formation. The soil moisture condition changes with time and may not be sufficient for subsequent nodulation and their potential activities. (Ramos *et al.*, 1999).

The ability of legumes to derive N through symbiotic N_2 fixation reduces their dependence on soil N for growth. However, several factors can affect N_2 fixation in legumes. Kirda *et al.* (1989) demonstrated that N_2 fixation was the most sensitive parameter to drought, followed by plant growth, and the least sensitive by soil N uptake. The N_2-fixing effectiveness of the legume-*Rhizobium* symbiosis has been estimated in various ways. Little is known about the effect of deficit irrigation scheduling on nodulation and N accumulation at different growth stages of uninoculated soybean.

Research Objectives

Based on the above description, a series of vinyl house with open surrounding sides experiments were conducted with soybean cultivar (*Glycine max L. Merrill*), located (35°27' N. and 136°44' E.) in the experimental farm of Gifu University, Japan, with the following objectives:

1. To evaluate the potentialities of the three soil types under various water deficit conditions to yield of soybean,
2. To elucidate the water stress effects on Soil Plant Analytical Development (SPAD) chlorophyll meter reading and its relationship to nitrogen status and grain yield of soybean under the three soil types,
3. To investigate the effects of water stress on root/shoot ratio, water use efficiency (*WUE*) and yield efficiency *(YE)* at different growth stages of soybean, and
4. To assess the effect of water stress on nodulation of uninoculated soybean and leaf N accumulation to grain yield at different growth stages of soybean.

2. Materials and Methods

2.1 Experiment I

2.1.1 Area description

This research was conducted in a vinyl house with open surrounding sides, located in the experimental farm of Gifu University (35°27' N. and 136°44' E.), Japan, from June to November 2007. The average temperature was 22.4^{0}C and the relative humidity was 67.5% during experiment duration.

2.1.2 Soil types description

The first factor of this experiment was three different soil types namely: Inceptisol, Ultisol and Andisol. The characteristics of the three soil types are shown in Tables 1 and 2. The Inceptisol, taken from a paddy field in Yanagido farm of Gifu University, was clay loam having the highest fine particles content in the form of clay and silt, and also the highest water content at field capacity and wilting point. The Ultisol, taken from Yanagido farm of Gifu University, was sandy clay loam and had the lowest organic matter content and the lowest total available moisture. The Andisol, taken from Minokamo farm of Gifu University, was sandy loam, locally named as “Kuroboku soil” and had the highest organic matter and total nitrogen contents.

Table 1.1: Physical properties of three soil types

Soil Types	Soil texture			Textural Class	Particle density (g/cm^3)	Bulk density (g/cm^3)	Total porosity (m^3/m^3)	Three phase distribution (m^3/m^3)		
	Sand (g/g)	Silt (g/g)	Clay (g/g)					Solid phase	Water Phase θ_{FC} (31kPa)	Air phase
Inceptisol	0.40	0.27	0.33	clay loam	2.61	1.06	0.60	0.40	0.39	0.21
Ultisol	0.59	0.18	0.23	sandy clay loam	2.65	1.37	0.48	0.52	0.32	0.16
Andisol	0.63	0.20	0.17	sandy loam	2.49	1.07	0.57	0.43	0.35	0.22

Table 1.2: Moisture properties of three soil types

Soil Types	Field Capacity θ_{FC} (31kPa) (m^3/m^3)	Wilting Point θ_{PWP} (1553kPa) (m^3/m^3)	Total Available Moisture $\theta_{FC}-\theta_{PWP}$ (m^3/m^3)
Inceptisol	0.390	0.228	0.162
Ultisol	0.323	0.182	0.141
Andisol	0.350	0.185	0.165

Table 1.3: Chemical properties of the three soil types

Soil Types	pH (H_2O)	Organic matter (g/g)	Total Carbon (g/g)	Total Nitrogen (g/g)	Available Phosphorus (g/kg)	Exchangeable Potassium (mg/100g)
Inceptisol	6.52	0.051	0.030	0.0021	0.060	31.6
Ultisol	6.70	0.020	0.012	0.0011	0.157	30.0
Andisol	6.25	0.095	0.055	0.0035	0.164	13.2

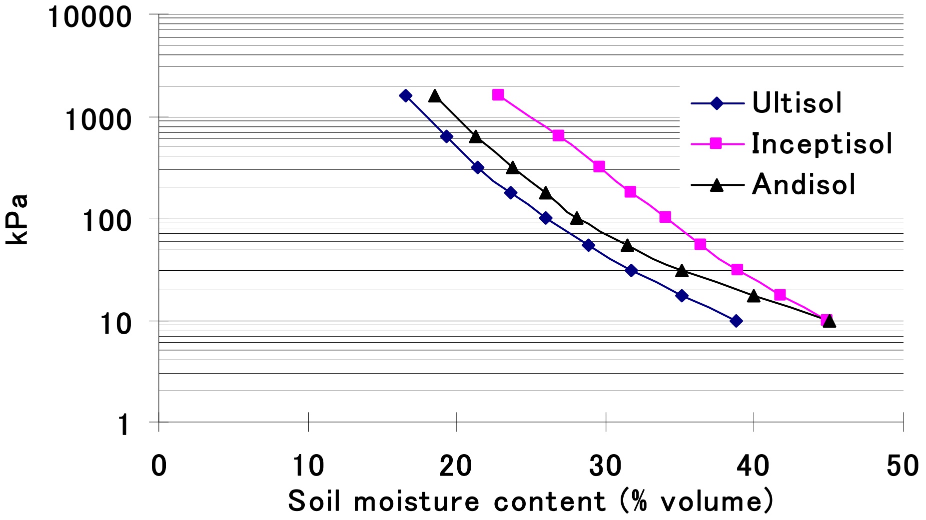


Figure 2.1: Moisture retention curve of the three soil types

2.1.3 Available soil water deficit level

The second factor was the water stress (*D*) with four levels of water deficit treatments imposed as D_1(0-25%), D_2(25-50%), D_3(50-75%) and D_4(75-100%) of available water deficit (Table 3). The D_2(25-50%) water deficit level for example, meant that the available water was maintained between 25% and 50% of total available water (*TAW*) throughout the growing season. When the maximum allowable depletion of available water came closer to 50% of *TAW*, water was applied to restore the available water to the deficit level of 25% of *TAW*. *TAW* is defined as the water content between field capacity (θ_{FC}) and permanent wilting point (θ_{PWP}).

2.1.4 Cultivation practices

Prior to planting, uniform amount of water was supplied to plastic pots of volume 10 liters and 23.8cm diameter filled with 10 kg air-dried soil to bring them to field capacity (θ_{FC}) for uniform germination. Then five seeds of soybean cultivar *Glycine max*

L. Merrill were planted in each pot. One week later, the emerged seedlings were thinned to only two seedlings, which were maintained until the end of the growth period. NPK fertilizer was applied just once during the seedling stage at a rate of 20 N, 180 P_2O_5 and 100 K_2O (kg/ha) respectively. The soil moisture for all pots was maintained at field capacity (θ_{FC}) until 21 days after sowing (DAS). After 21 DAS, the deficit irrigation treatments were initiated. The irrigated period of soybean was 22 weeks from June 9 to November 9. The pot served as the role of a weighing lysimeter that hydrologically isolates soil surface lateral inflow/outflow.

2.1.5 Agronomic variables

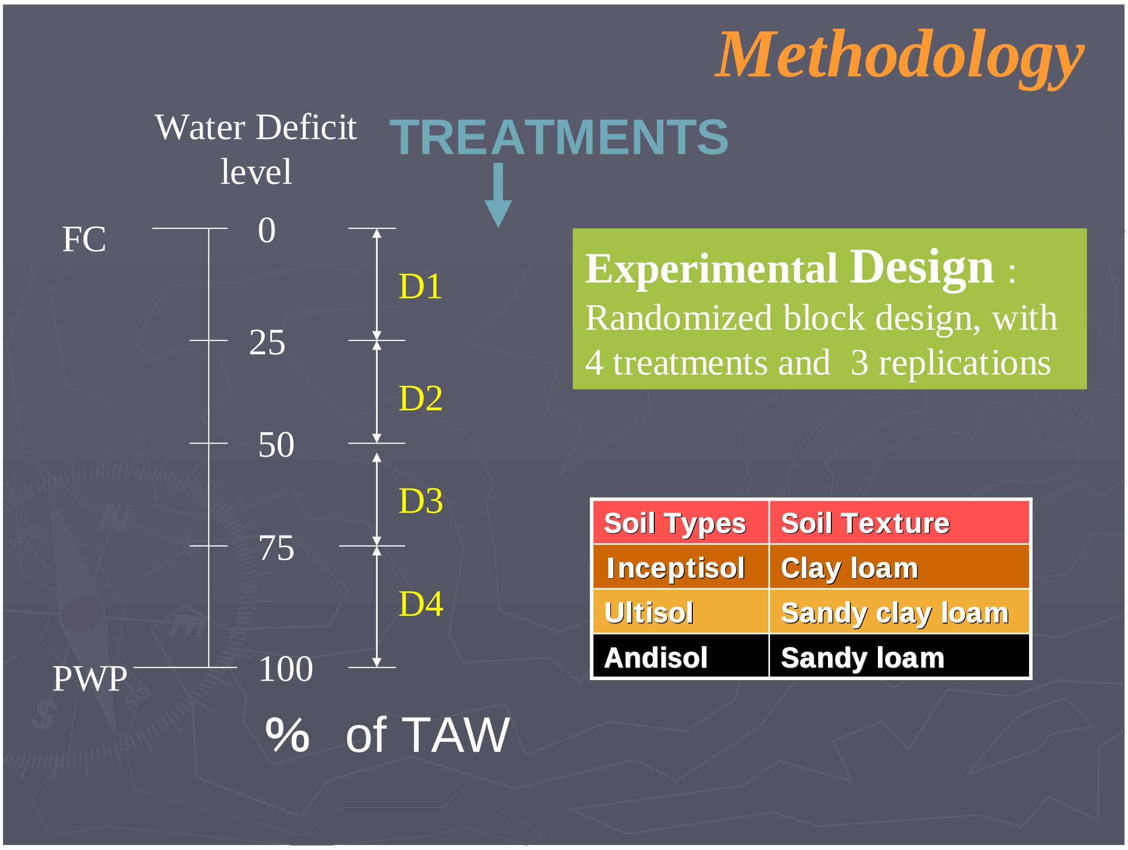
Agronomic variables evaluated in this research were crop water requirement (*CWR*, g/pot), oven dry (at 65°C for 96 h) weight of total biomass including roots (*TDB*, g/pot) and air-dried grain yield of soybean (*Y*, g/pot), and leaf area index (*LAI*, m^2/m^2). *LAI* was measured at 84 DAS according to Fehr and Cavines (1977) using a portable leaf area meter (Model LI 3000A; LI-COR Inc. Lincoln, NE, USA) from each pot. Crop water requirement (*CWR*, g/pot) was calculated from the evapotranspiration during the irrigated period of soybean according to Allen *et al.* (1998). Daily evapotranspiration (*ET*) was measured by weighing the pot every day.

2.1.6 Leaf N and grain N status

The harvested soybean leaves were dried at 80^oC for 48 hours, grain yield was air-dried, and after that, ground samples were screened through 1 mm sieve. Then the leaf N and grain N status were determined with an automatic high sensitive NC analyzer (Sumigraph NC 95A, Shimadzu Co. Ltd., Japan).

2.1.7 SPAD chlorophyll meter reading (SCMR)

SPAD chlorophyll meter (Minolta SPAD-502 meter, Tokyo, Japan) readings were taken weekly at the surface close to the mid–rib of the youngest fully expanded leaf. Based on the recommendation by Costa *et al.* (2003), thirty leaves were measured at random and an average SPAD value was calculated for each pot. The Minolta SPAD 502 meter collects and stores up to 30 individual readings and calculates the average automatically. The results of SPAD chlorophyll meter readings (SCMR) at seed formation stage (84 DAS) are presented, because leaf chlorophyll reaches its maximum at that time.



Methodology

Experimental Design :
Randomized block design, with
4 treatments and 3 replications

Soil Types	Soil Texture
Inceptisol	Clay loam
Ultisol	Sandy clay loam
Andisol	Sandy loam

Figure 2.2: Treatments of the experiment conducted in the year 2007 (4 water deficit levels x 3 different soil types x 3 replications = 36 pots).

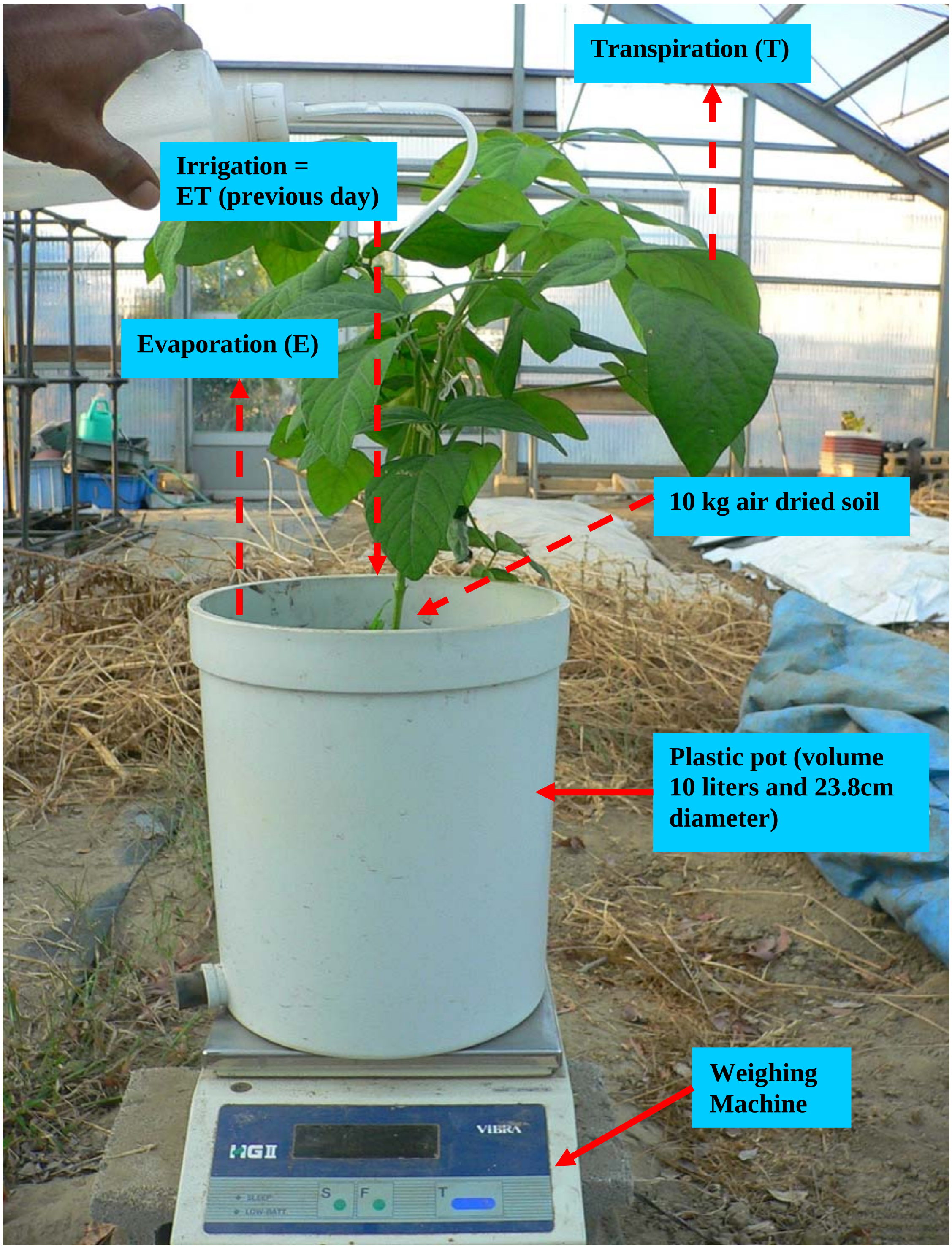


Figure 2.3: Daily evapotranspiration (*ET*) was measured and water was applied to maintain the water deficit level as per treatment by weighing the soybean plant with plastic pot every day.

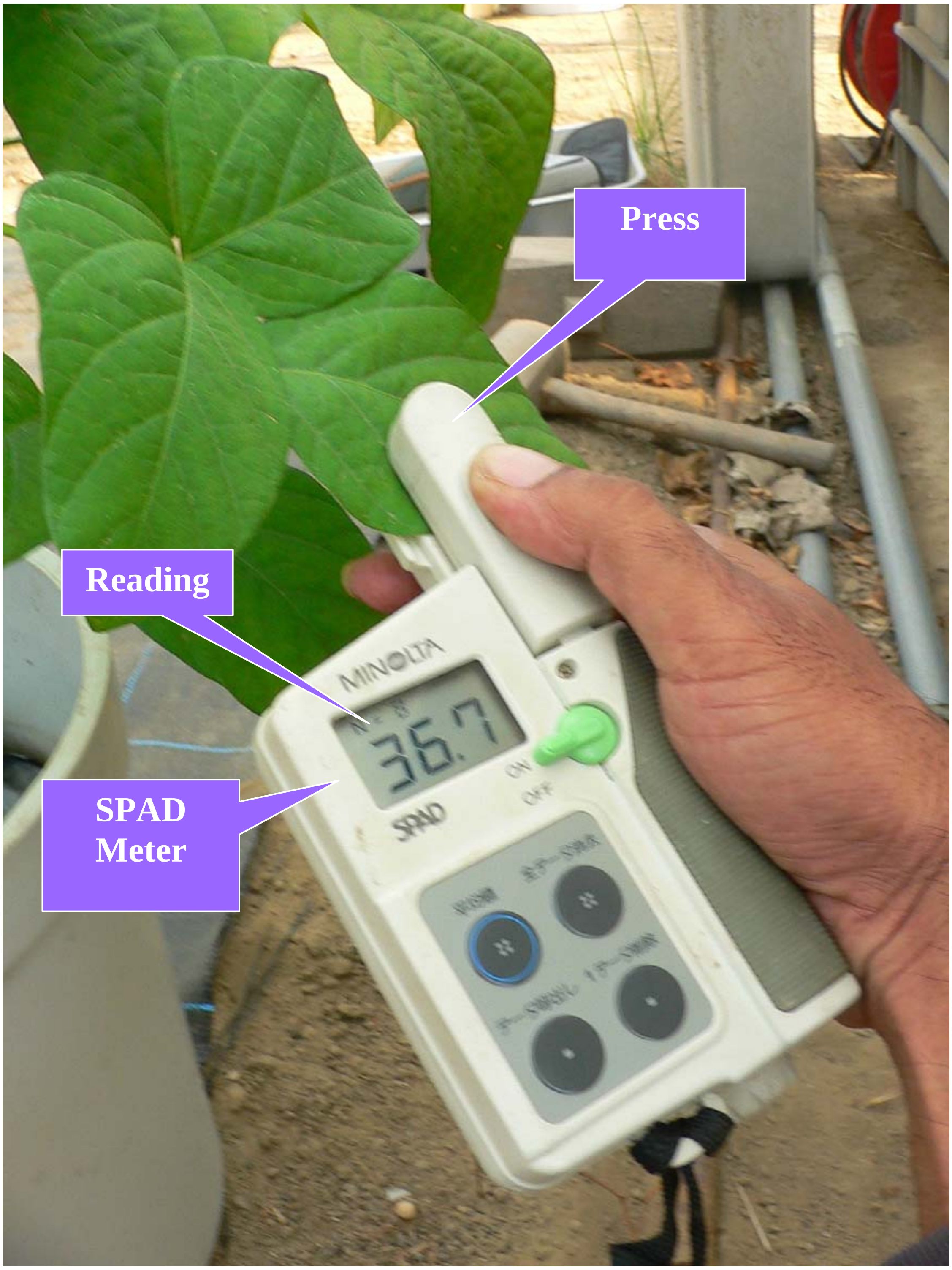


Figure 2.4: Soil Plant Analytical Development (SPAD) chlorophyll meter readings (SCMR) were taken weekly at the surface close to the mid–rib of the youngest fully expanded leaf of the soybean plant.

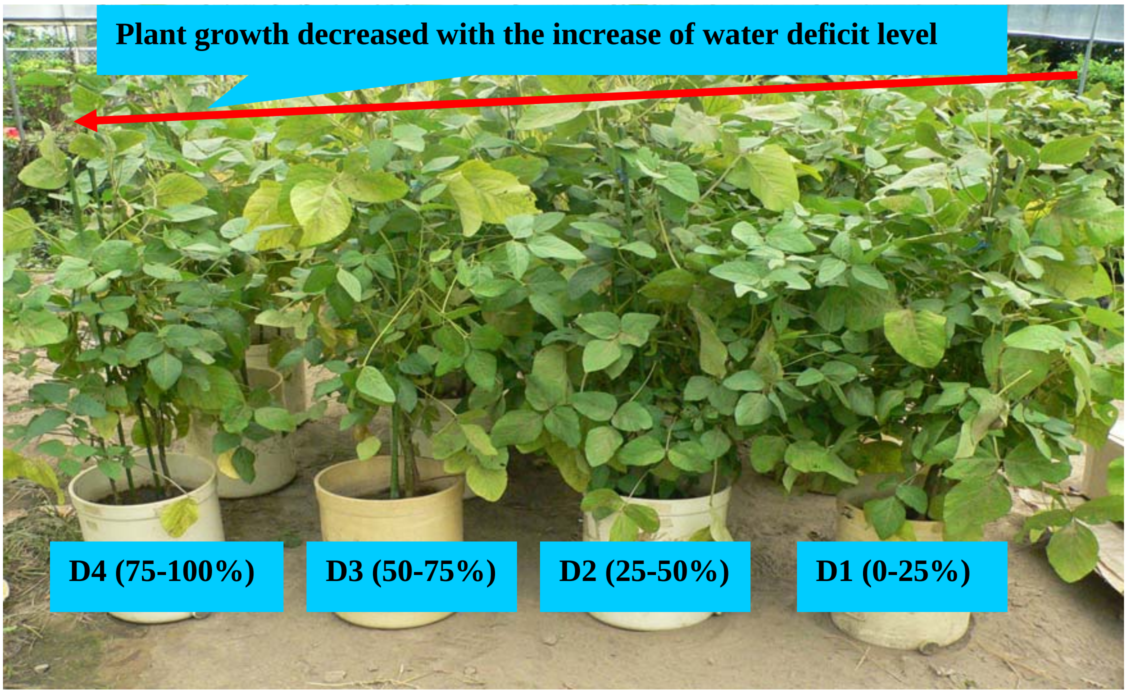


Figure 2.5: Soybean plant (112 DAS) under water deficit conditions in Inceptisol (4 treatments with 3 replications).

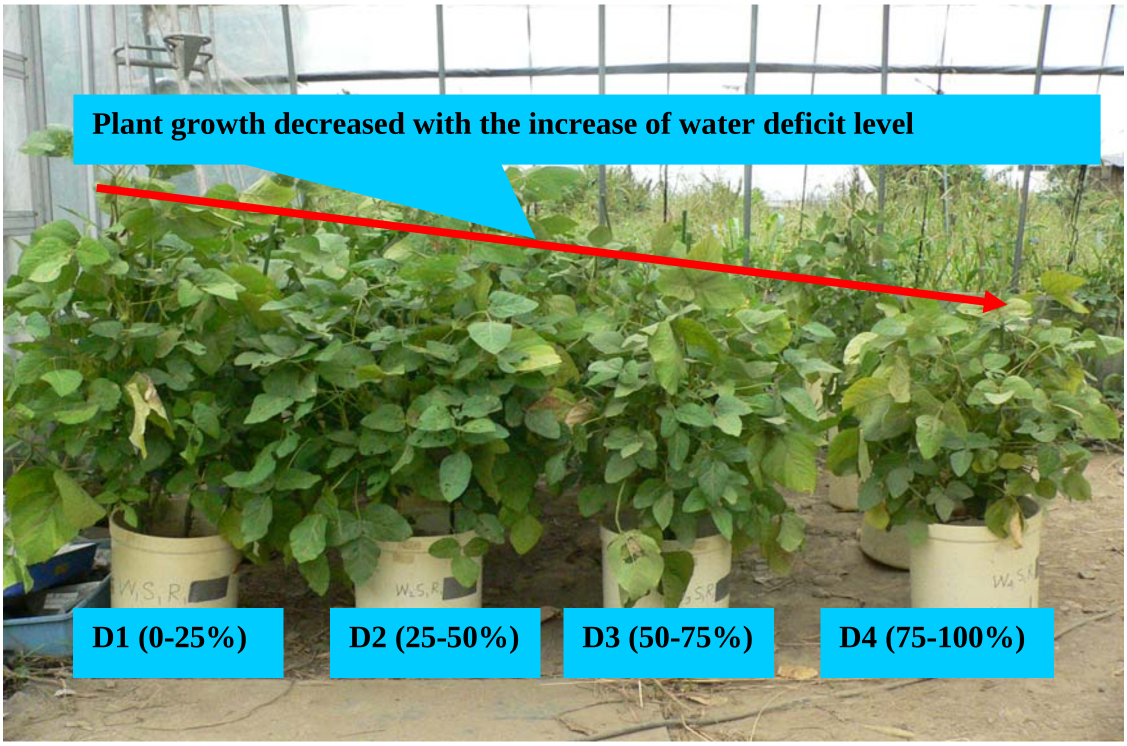


Figure 2.6: Soybean plant (112 DAS) under water deficit conditions in Ultisol (4 treatments with 3 replications).

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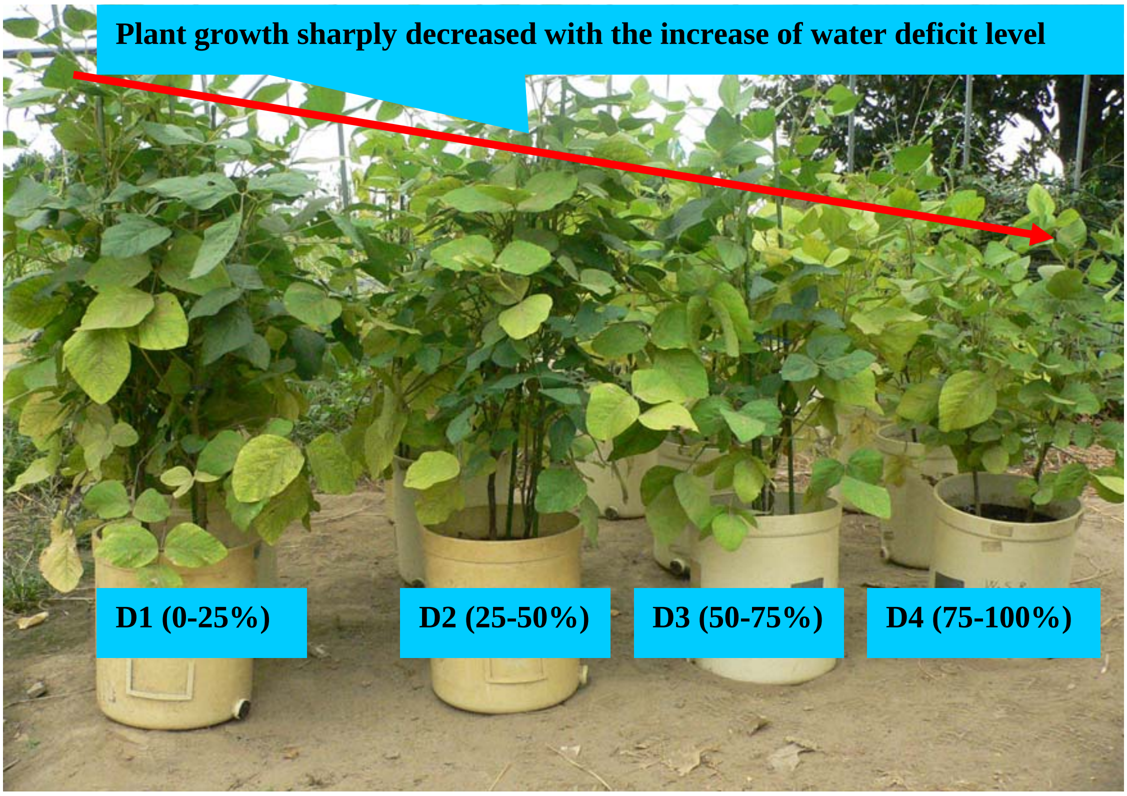

Figure 2.7: Soybean plant (112 DAS) under water deficit conditions in Andisol (4 treatments with 3 replications).

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2.2 Experiment II

2.2.1 Area description

This research was conducted in a vinyl house (surrounding sides were open) located in the experimental farm of Gifu University (35°27' N. & 136°44' E.), Japan, from June to November 2008. The average temperature was 22.4℃ and the relative humidity was 67.5% during experiment duration. The soil was clay loam in texture (0.40g/g sand, 0.27g/g silt and 0.33g/g clay) and classified as Inceptosl. The bulk density was 1.07 (g/cm^3). Soil water content at field capacity (34.7kPa) was 0.516 m^3/m^3 and wilting point (185kPa) was 0.296 m^3/m^3. Therefore, the total available water (*TAW*) was 0.220 m^3/m^3.

Table 2.1: The soil physical, moisture and chemical properties

Physical properties	Texture (g/g)	sand: 0.40 silt: 0.27 clay: 0.33
	Textural class	clay loam
	Particle density (g/cm^3)	2.49
	Bulk density:(g/cm^3)	1.07
	Total porosity (m^3/m^3)	0.57
Moisture properties	Field capacity, θ_{FC} (31kPa) (m^3/m^3)	0.516
	Wilting point, θ_{PWP} (1553kPa) (m^3/m^3)	0.296
	Total available moisture, $\theta_{FC} - \theta_{PWP}$	0.220
Chemical properties	pH (H_20)	6.41
	Organic matter (g/g)	0.065
	Total carbon (g/g)	0.038
	Total nitrogen (g/g)	0.0026
	C/N	13.3
	Available phosphorus (g/kg)	0.164
	Exchangeable potassium (mg/100g)	13.2

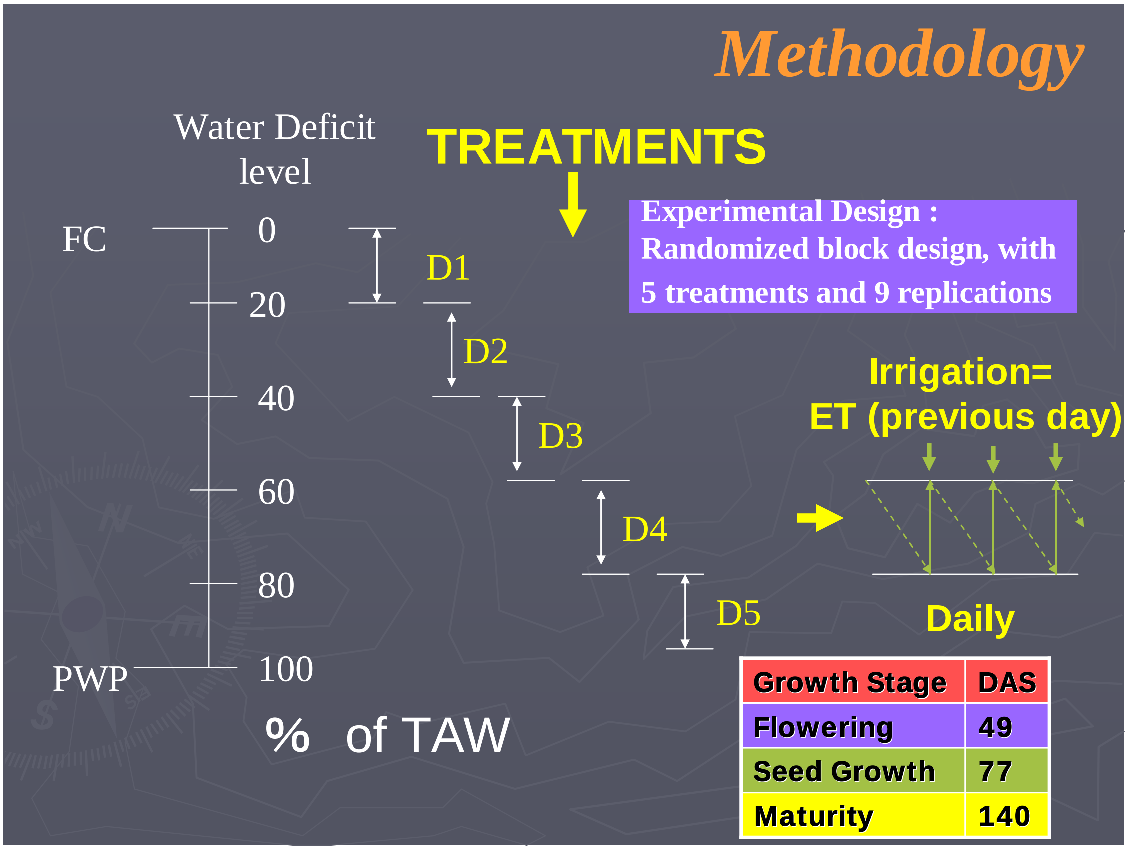
2.2.2 Treatments and experimental design

Five water deficit treatments namely; $D1$ (0-20%), D_2 (20-40%), D_3 (40-60%), D_4 (60-80%) and D_5 (80-100%) of total available water deficit (TAW) were arranged in a completely randomized block design with nine replications. The water deficit level of D_2 (20-40%), for example, meant that the available water was maintained between 20% and 40% of TAW throughout the growing season. When the maximum allowable depletion of available water came closer to 40% of TAW, water was applied to restore the available water to the deficit level of 20% of TAW. The TAW is defined as the water content between field capacity (θ_{FC}) and permanent wilting point (θ_{PWP}).

Plastic pots (10 liters volume and 23.8cm diameter) with no drainage holes were filled with 7 kg air-dried Inceptisol (clay loam in texture). Then five soybean seeds [*Glycine max* (L.) Merrill] were sown in each pot. Prior to planting, uniform water was applied to all the pots to bring them to field capacity (θ_{FC}) for uniform germination. The soil moisture for all pots was maintained at field capacity (θ_{FC}) until 14 days after sowing (DAS). After 14 DAS, the deficit irrigation treatments were initiated. The irrigated period of soybean was 20 weeks from June 16 to November 3. The plants were thinned to one per pot at the 2- to 3- leaf stage. Three replicate pots of each water deficit level were sampled at 49 DAS (flowering stage), 77 DAS (seed growth stage), and 140 DAS (maturity stage) during the experiment. Three plants per treatment were used for final yield analyses.

2.2.3 Measurements

Agronomic variables evaluated in this research were crop water requirement (CWR, g/plant), oven dry (at 65°C for 96 h) weight of total biomass including roots (TDB, g/plant), root dry weight (g/plant), shoot (leaves and stem) dry weight (g/plant) and air-dried grain yield of soybean (Y, g/plant). Leaf area index (LAI, m^2/m^2) were measured according to Fehr and Cavines (1977) using a portable leaf area meter (Model LI 3000A; LI-COR Inc. Lincoln, NE, USA) from each pot. Crop water requirement (CWR, g/pot) was calculated from the evapotranspiration during the irrigated period of soybean according to Allen *et al.* (1998). Daily evapotranspiration (ET, mm/d) was measured by weighing the pot every day.



Methodology

Water Deficit
level

TREATMENTS

Experimental Design :
Randomized block design, with
5 treatments and 9 replications

FC

0

20

40

60

80

100

PWP

D1

D2

D3

D4

D5

% of TAW

Irrigation=
ET (previous day)

Daily

Growth Stage	DAS
Flowering	49
Seed Growth	77
Maturity	140

Figure 2.8: Treatments of the experiment conducted in the year 2008.

Plants were harvested in a laboratory so that nodule fresh weights (NFW) could be recorded immediately. Soil was removed from plant roots, and nodules were separated from the roots. Nodules were sorted using 4.75-mm wire-mesh sieves resulting in two nodule diameter size classes (≥4.75 mm and ≤ 4.75 mm). The NFW and the number of nodules per plant were recorded according to the two diameter size classes. All plant tissues (leaves, stem, root, and nodules by size class) were dried at 65°C for 96 h and dry weights recorded.

Then ground samples of dried soybean leaves were screened through 1 mm sieve. The leaf N status was determined with an automatic high sensitive NC analyzer (Sumigraph NC 95A, Shimadzu Co. Ltd., Japan).

Figure 2.9: Flowering stage of soybean plant at 49 DAS.

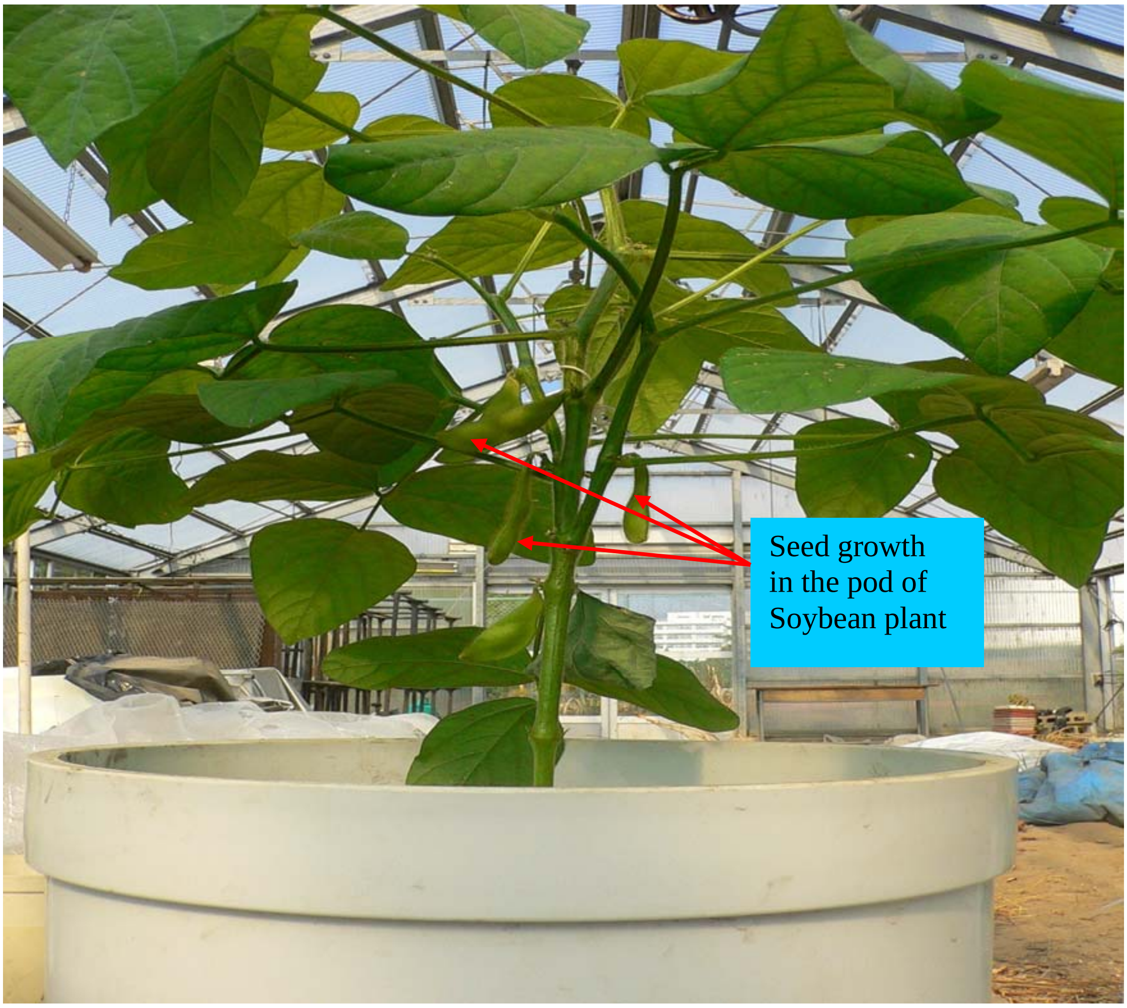


Figure 2.10: Seed growth stage in the pod of soybean plant at 77 DAS.

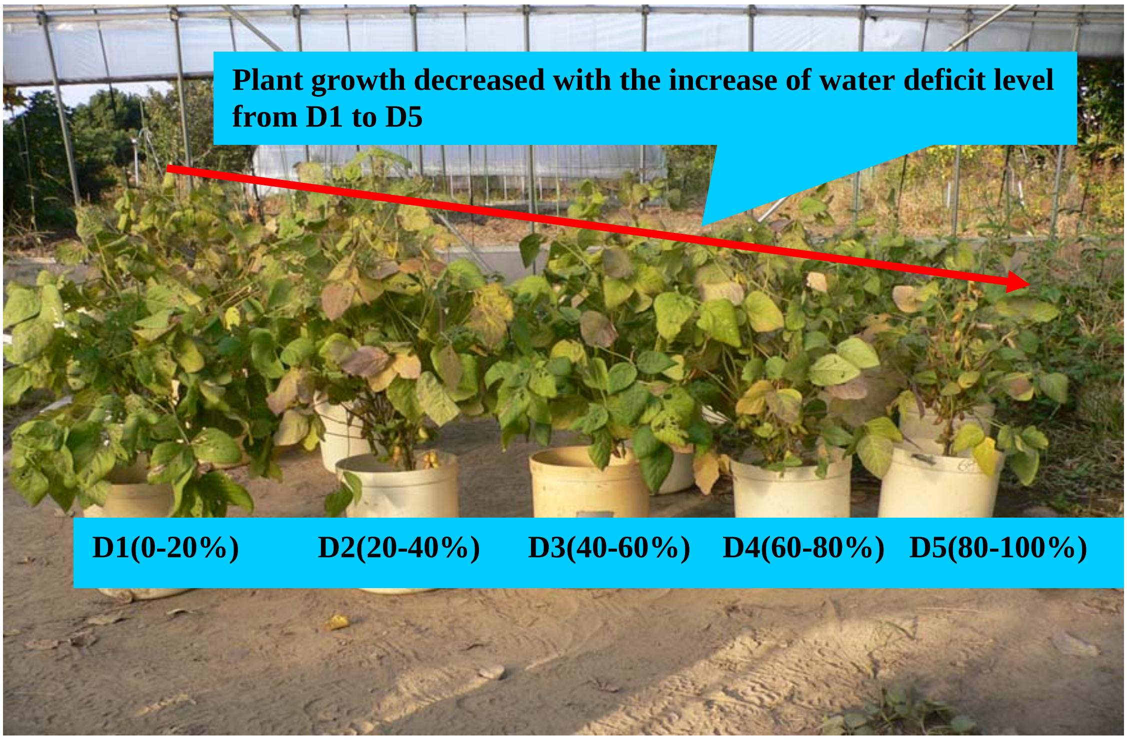


Figure 2.11: Maturity stage of soybean plant (140 DAS) under water deficit conditions.

D1(0-20%) D2(20-40%) D3(40-60%)

D4(60-80%) D5(80-100%)

Figure 2.12: Soybean plant under different water deficit levels at maturity stage.

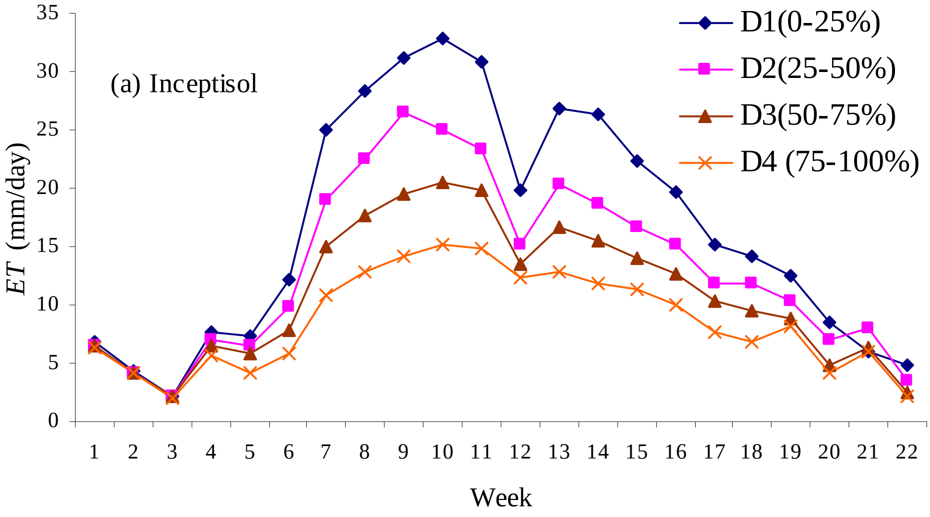
Figure 2.13: Nodule formation of the soybean plant root at 28 DAS.

3. Results and Discussions

3.1 Evaluation of the Potentialities of Different Soil Types to Yield Response of Soybean under Deficit Irrigation

3.1.1 Crop water requirement (*CWR*) and water stress coefficients (K_s)

The evapotranspiration (*ET*, mm/day) of soybean plant in each soil type Inceptisol (a), Ultisol (b), and Andisol (c) decreased with the increasing water deficit levels imposed (Figure 3.1.1). Table 3.1.1 also shows that crop water requirement (*CWR*) in each soil type significantly decreased with the increasing water deficit levels. Furthermore, when the *CWR* under each of the corresponding water deficit levels was compared among the three soil types, the following trend was observed: Inceptisol>Ultisol>Andisol in that order. Therefore, the above *CWR* relationship among the three soil types indicates that plants can undergo water stress quickly in Andisol which is coarse textured (sandy loam) soil, whereas plants in the finer textured Inceptisol (clay loam) have ample time to adjust to low soil water matric pressure, and may remain unaffected by water stress. Tables 1.1 and 1.2 support the above explanation.



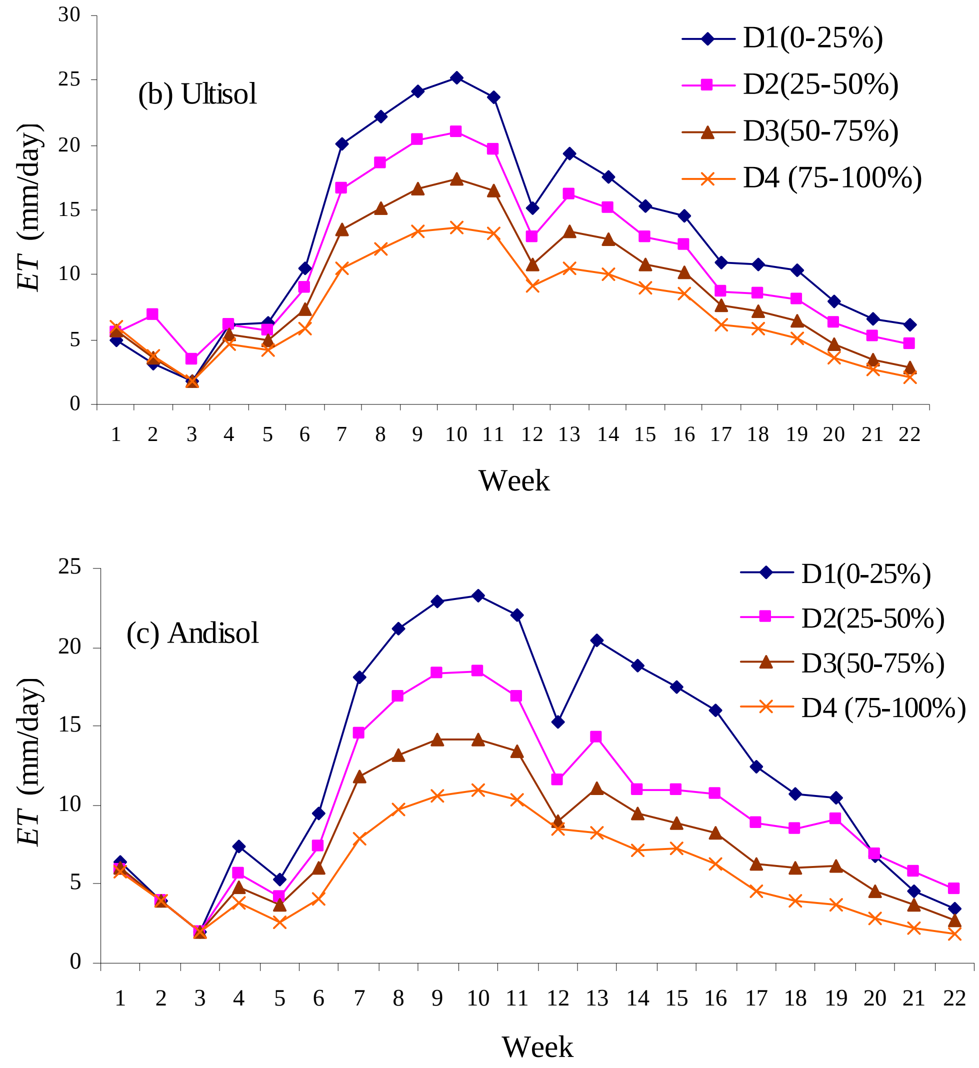


Figure 3.1.1: Evapotranspiration (*ET*,mm/day) of soybean plant in Inceptisol (a), Ultisol (b), and Andisol (c).

Figure 3.1.2 shows that *CWR* linearly correlated with leaf area index (*LAI*) without differences among the three soil types. Based on this result, it can be said that among all the agronomic factors, *LAI* as a growth indicator was the most sensitive in the control of evapotranspiration rate. This result agrees with Setiyono *et al*. (2008) who found the same phenomenon that transpiration is directly controlled by leaf area index.

Table 3.1.1: The effect of water deficit level on crop water requirement (*CWR*), leaf area index (*LAI*), total dry biomass, grain yield, water use efficiency (*WUE*) and yield efficiency (*YE*) of soybean under different soil types

Soil Types	Water Deficit Level (%)	*CWR* (g/pot)	*LAI* (m^2/m^2)	Total dry biomass (g/pot)	Grain yield (g/pot)	*WUE* (g/g)	*YE* (g/g)
		①	②	③	④	⑤=③/①	⑥=④/①
Inceptisol	D_1 (0- 25)	116,504A a	5.1 A a	214 A a	31.8 A a	0.00184 AB c	0.000273 B c
	D_2 (25- 50)	92,949 A b	4.7 A b	176 A b	29.5 A b	0.00189 A b	0.000317 A b
	D_3 (50- 75)	76,794 A c	4.1 A c	146 A c	26.7 A c	0.00190 A a	0.000348 A a
	D_4 (75-100)	60,608 A d	3.1 A d	121 A d	18.8 A d	0.00200 A a	0.000310 A b
Ultisol	D_1 (0- 25)	90,034 B a	4.6 B a	172 B a	27.4 B a	0.00191 A b	0.000304 A b
	D_2 (25- 50)	77,877 B b	4.2 B b	151 B b	25.4 B b	0.00194 A b	0.000326 A a
	D_3 (50- 75)	63,442 B c	3.3 B c	124 B c	21.4 B c	0.00195 A b	0.000337 A a
	D_4 (75-100)	51,980 B d	2.3 B d	106 B d	14.8 B d	0.00204 A a	0.000285 B c
Andisol	D_1 (0- 25)	88,883 B a	4.4 B a	160 C a	26.6 B a	0.00180 B a	0.000299 A a
	D_2 (25- 50)	68,961 C b	3.5 C b	128 C b	21.5 C b	0.00186 A a	0.000312 A a
	D_3 (50- 75)	54,355 C c	2.5 C c	102 C c	17.5 C c	0.00188 A a	0.000332 A a
	D_4 (75-100)	40,919 C d	0.8 C d	74 C d	6.7 C d	0.00181 A a	0.000164 C b

Means followed by different small letters (a-d) in the same column in each soil types under different water deficit levels are significantly different according to Tukey's multiple comparison test ($p<0.05$).

Means followed by different capital letters (A-C) vertically at same water deficit level among the three soil types are significantly different according to Tukey's multiple comparison test ($p<0.05$).

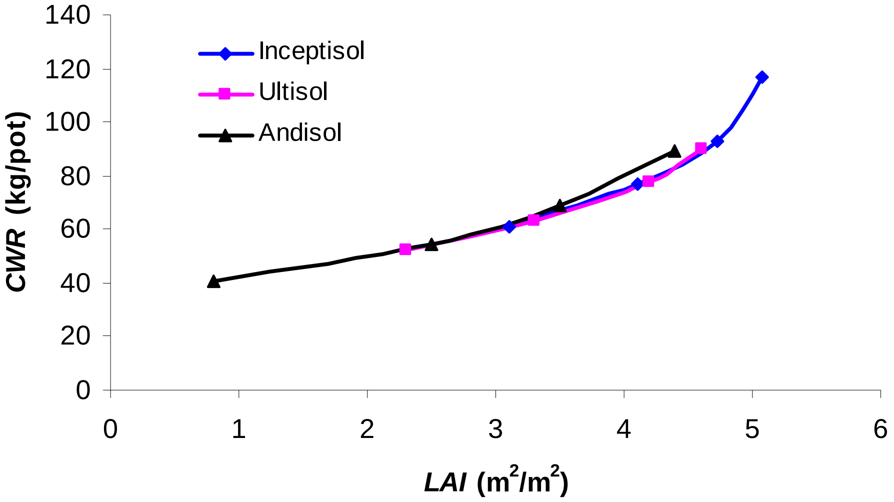


Figure 3.1.2: The relation between crop water requirement (*CWR*) and leaf area index (*LAI*).

According to Allen *et al.* (1998), evapotranspiration under water stress condition is referred to as the adjustment evapotranspiration (ET_{cadj}, mm/d) which can be calculated by the following equation.

$$ET_{cadj}=K_s\,ET_c \qquad (1)$$

where ET_c (mm/d) is the crop evapotranspiration under standard condition, K_s *is* water stress coefficient (no dimension).

The value of K_s is important for estimating ET_{cadj}, and can be used for deficit irrigation scheduling. K_s describe the effect of water stress on crop transpiration (Allen *et al.* 1998). Assuming that the evapotranspiration at D_1(0-25%) occurred under the ideal condition for plant growth in which the soil water content is near the field capacity, the actual evapotranspiration (ET_a) at D_1 is crop evapotranspiration (ET_c), which means the evapotranspiration of plant under standard conditions (Allen *et al.*, 1998). Water stress coefficient (K_s) is calculated as the ratio between the actual evapotranspiration (ET_a) at each water deficit level and the crop evapotrarspiration (ET_c). The ratio of water depletion to the total available water in the root zone, referred to as “p”, is an indicator of the water deficit level. For example, the average value of “p” under the water management of D_2 (25-50%) treatment is calculated as “p” = (0.25+0.50)/2=0.38.

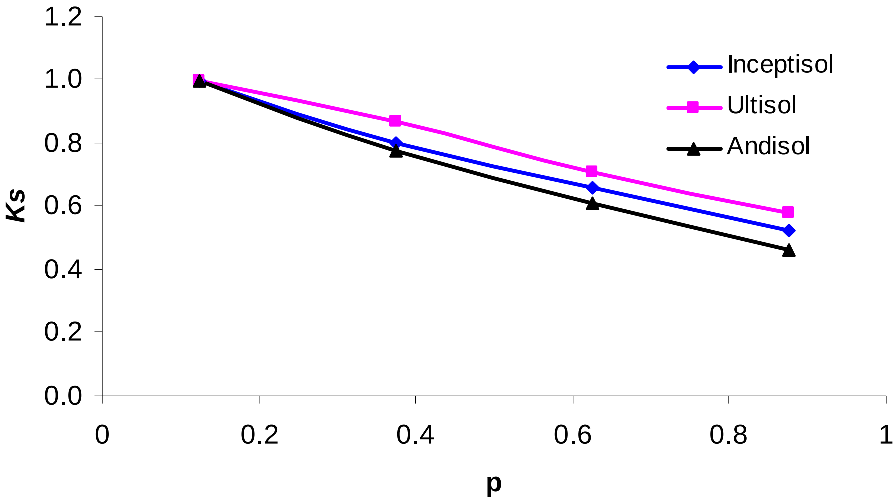


Figure 3.1.3: The effect of available water deficit (p) on water stress coefficient (K_s).

Figure 3.1.3 shows that the K_s value decreased linearly with the increase of available water deficit level “p”. It indicates that the K_s value (water stress coefficients) in Andisol is more sensitive to water deficit than the other two soil types. This is mainly due to the coarse textured nature of Andisol **(Table 1.1).**

3.1.2 Total dry biomass (*TDB*) and water use efficiency (*WUE*)

Table 3.1.1 shows that total dry biomass (*TDB*, g/pot) of soybean in each of the three soil types significantly decreased with the increase of water deficit levels. Furthermore, the *TDB* of soybean in the three soil types under each of the corresponding water deficit levels, significantly decreased in the order of Inceptisol> Ultisol> Andisol. The *TDB* of the three soil types linearly correlated with *CWR* under the water deficit levels (Figure 3.1.4). This result indicated that the decrease in total dry biomass was due to the considerable reduction in plant growth and canopy structure caused by the water stress conditions. This phenomenon agrees with Hong-Bo Shao *et al.* (2008) who found that the biomass of soybean plant was reduced by the water stress imposed.

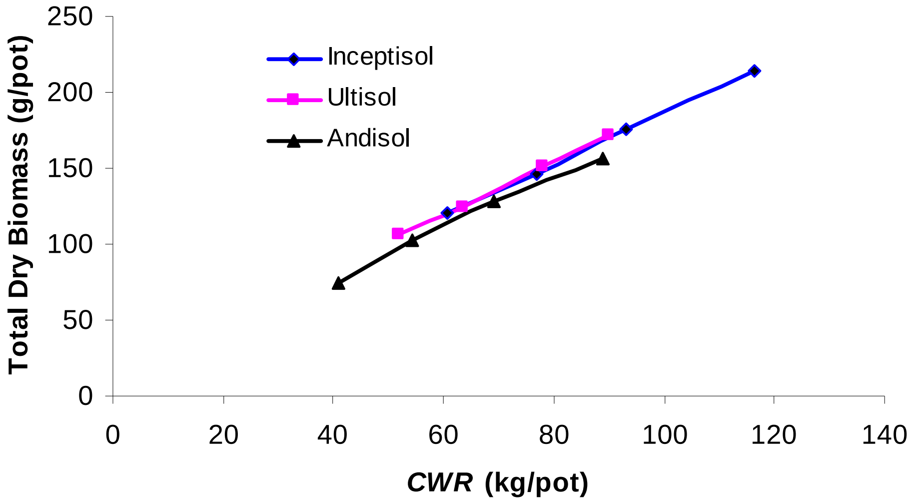


Figure 3.1.4: The relation between total dry biomass and crop water requirement (*CWR*).

Water use efficiency (*WUE*, g/g) is defined as the ratio of total dry biomass (*TDB*, g/pot) to the crop water requirement (*CWR*, g/pot). Table 3.1.1 shows that the *WUE* value slightly increased with the increase of water deficit level, except water deficit level D_4 in Andisol. Consequently, the highest *WUE* value was obtained at the water deficit level D_4 in Ultisol and Inceptisol, while the highest *WUE* in Andisol was at water deficit level D_3. However, there was no difference at 5% significant level among the *WUE* values of the same water deficit level from D_2 to D_4 in the three soil types. This result indicated that there was little influence of the soil types on *WUE* value at the same water deficit level.

3.1.3 Grain yield and yield efficiency (*YE*)

The grain yield of soybean in the three soil types decreased with the increase of water deficit levels (Table 3.1.1). Similar to *TDB*, the grain yield of soybean in the three soil types under each of the corresponding water deficit levels, significantly decreased in the order of Inceptisol > Ultisol > Andisol. Figures 3.1.5 and 3.1.6 shows that the grain yield of soybean was strongly influenced by both *CWR* and *LAI* among the three soil types, respectively. These results indicated that the reduction in *CWR* by water stress caused the decrease of soil water uptake with soluble nutrients and consequently the decrease of soybean grain yield through reduction in photosynthesis.

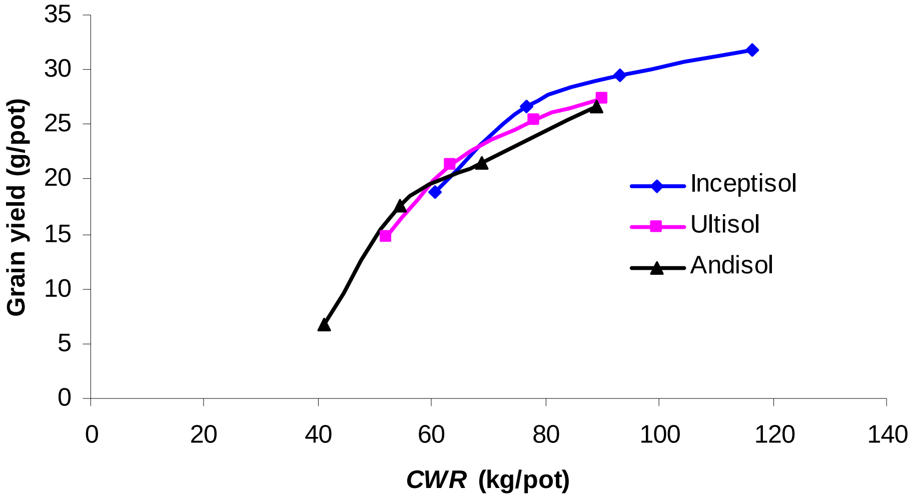


Figure 3.1.5: The relation between the grain yield and crop water requirement (*CWR*).

Yield efficiency (*YE*, g/g) is defined as the ratio of grain yield (*Y*, g/pot) to crop water requirement (*CWR*, g/pot). There was an effect of water deficit (*D*) on the *YE* value in the three soil types at 5% significant level (Table 3.1.1). Table 3.1.1 shows that the *YE* value slightly increased with the increase of water deficit level from D_1 to D_3. However, there was no significant difference at 5% level among the *YE* values of the three soil types at the water deficit level D_3. These results indicated that soil moisture and aeration at the water deficit level D_3 were the most appropriate for maximizing the *YE* value, and the maximum values of *YE* were slightly influenced by the soil types. On the other hand, significant differences appeared among the *YE* values at the water deficit levels D_1 and D_4 of the three soil types. The smallest *YE* value at full irrigation (D_1) was shown in Inceptisol, which contained the finest soil texture, probably due to lack of aeration in the soil. On the other hand, the smallest *YE* value at the deficit irrigation which controlled soil moisture near the wilting point (D_4) was in Andisol with the coarsest soil texture probably due to excessive water stress.

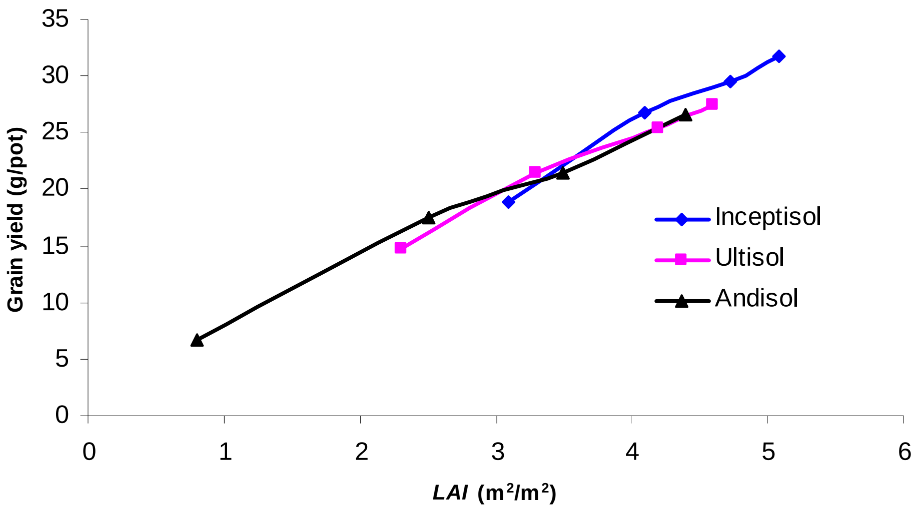


Figure 3.1.6: The relation between the grain yield and leaf area index (*LAI*).

3.1.4 Yield response factor (K_y)

According to Doorenboss and Kassam (1979), in order to quantify the effect of water stress, it is necessary to derive the relationship between relative yield decrease and relative evapotranspiration deficit given by the following equation.

$$1-\frac{Y_a}{Y_m}=K_y\times(1-\frac{ET_a}{ET_m}) \qquad (2)$$

where 1-Y_a/Y_m: relative yield decrease, Y_a: actual yield, Y_m: maximum yield (under no stress condition), 1-ET_a/ET_m: relative evapotranspiration decrease, K_y: yield response factor, ET_a: actual evapotranspiration, and ET_m: maximum evapotranspiration

Under conditions of limited water distributed equally over the total growing season, involving crops with different K_y values, the crop with higher K_y value will suffer a greater yield loss than the crop with a lower K_y value (Moutonnet, 2000). The K_y values for most crops are derived on the assumption that the relationship between relative yield (Y_a/Y_m) and relative evapotranspiration (ET_a/ET_m) is linear and valid for water deficit of up to about 50 percent or 1- ET_a/ET_m =0.5 (Kirda *et al.*,1999). According to a report by Doorenboss and Kassam (1979), the K_y of soybean under water deficit for the whole growing period was found to be 0.85.

Table 3.1.2: The effect of water deficit level on water stress coefficient (K_s) and yield response factor (K_y) of soybean under different soil types

Soil Types	Water Deficit Level (%)	Yield (g/pot)	ET_a (g/pot)	$K_s = \frac{ET_a}{ET_m}$	$1-\frac{Y_a}{Y_m}$	$1-\frac{ET_a}{ET_m}$	K_y
Inceptisol	D_1 (0- 25)	31.8	116504	1	0	0	-
	D_2 (25- 50)	29.5	92949	0.80	0.07	0.20	0.36
	D_3 (50- 75)	26.7	76794	0.66	0.16	0.34	0.47
	D_4 (75-100)	18.8	60608	0.52	0.41	0.48	0.85
Ultisol	D_1 (0- 25)	27.4	90034	1	0	0	-
	D_2 (25- 50)	25.4	77877	0.86	0.07	0.14	0.54
	D_3 (50- 75)	21.4	63442	0.70	0.22	0.30	0.74
	D_4 (75-100)	14.8	51980	0.58	0.46	0.42	1.09
Andisol	D_1 (0- 25)	26.6	88883	1	0	0	-
	D_2 (25-50)	21.5	68961	0.78	0.19	0.22	0.86
	D_3 (50-75)	17.5	54355	0.61	0.34	0.39	0.88
	D_4 (75-100)	6.7	40919	0.46	0.75	0.54	1.39

Y_a : actual yield, Y_m : maximum yield = yield under no water stress condition (D_1), ET_a : actual evapotranspiration, ET_m : maximum evapotranspiration = evapotranspiration under no water stress condition (D_1)

The K_y values of soybean in the three soil types, calculated by using the above equation (2), are shown in Table 3.1.2. The smallest K_y value was in Inceptisol, followed by Ultisol and then Andisol under all water deficit levels. Deficit irrigation in Inceptisol was effective (K_y<1.0) for economic water usage under all water deficit levels. On the other hand, the deficit irrigation in both Ultisol and Andisol was effective (K_y<1.0) under the water deficits lower than 50-75 % of *TAW* (D_3).

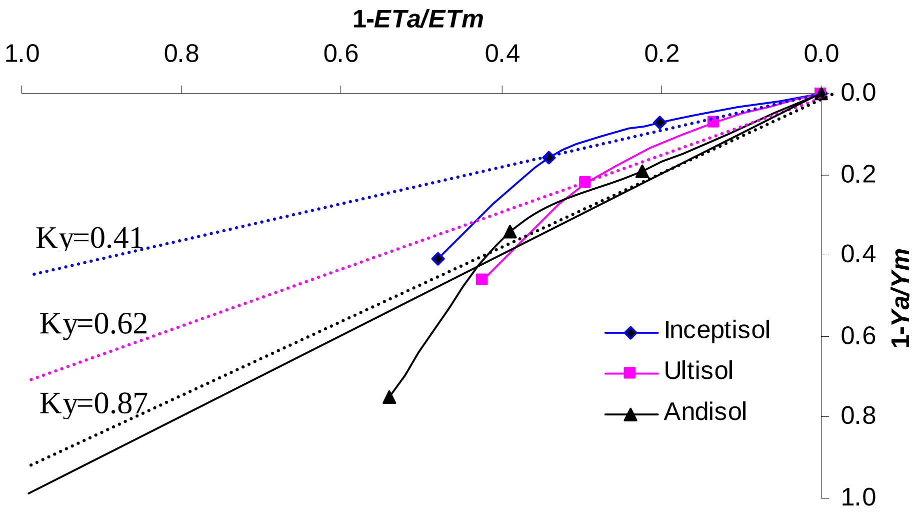


Figure 3.1.7: Yield response factor (K_y) for water deficit of soybean under three soil types.

The relative yield ($1-Y_a/Y_m$) linearly decreased with the relative water deficit ($1-ET_a/ET_m$) up to the D_3 water deficit levels (50-75% of *TAW*) and thereafter, greatly decreased form D_3 to D_4 water deficit level among the three soil types, and the mean value of K_y was 0.41, 0.62, and 0.87 in Inceptisol, Ultisol, and Andisol, respectively (Figure 3.1.7). However, the above results indicated that the K_y values of soybean were strongly influenced by soil physical properties, especially soil texture. The response of water stress to soybean grain yield was the smallest in fine-textured soil like Inceptisol (K_y=0.41), and was the greatest in coarse-textured soil like Andisol (K_y=0.87). It can be concluded from these results that the effect of deficit irrigation for saving irrigation water was great in Inceptisol with fine soil texture, followed by Ultisol with medium soil texture, and then Andisol with coarse soil texture.

3.1.5 Optimum deficit irrigation

The highest grain yield of soybean per unit area was produced under the full irrigation (D_1) in all the three soil types. The highest grain yield of soybean (*Y*, g/pot) at full irrigation was obtained in Inceptisol (*Y*=31.8g/pot), followed by Ultisol (*Y*=27.4g/pot), and then Andisol (*Y*=26.6g/pot). On the other hand, the optimum grain yield of soybean with the highest yields efficiency (*YE*) was obtained by the deficit irrigation, in which water deficit level was maintained at 50-75% of *TAW* (D_3). The water stress coefficient (K_s) at D_3 was 0.66, 0.70, and 0.61 in Inceptisol, Ultisol, and Andisol, respectively. The *YE* value at water deficit level D_3 was 1.27 times as much as that under the full irrigation (D_1) in the Inceptisol, and 1.11 times those of both Ultisol and Andisol. It was observed that the grain yield of soybean per unit area under deficit irrigation at 50-75% of *TAW* (D_3) was reduced by 16.0, 21.9, and 34.2 %, but could conserve 21.6, 9.8 and 9.9% of irrigation water to produce the same yield compared to the full irrigation (D_1) in the Inceptisol, Ultisol and Andisol, respectively.

3.2 Effects of Water Stress on Soil Plant Analytical Development (SPAD) Chlorophyll Meter reading and its Relationship to Nitrogen Status and Grain Yield of Soybean under Different Soil Types

3.2.1 *ET* and *LAI* contributing to grain yield

The *ET* and *LAI* within each soil type significantly decreased with the increasing water deficit levels imposed from D_1 to D_4 (Table 3). The Inceptisol had the highest *ET* and *LAI*, followed by Ultisol and then Andisol under all water deficit levels. The *ET* correlated almost linearly (R^2=0.9278) with *LAI* of soybean under the three soil types in response to water deficit levels (Figure 3.2.1). In addition, Figure 3.2.2 shows an almost a linear relation (R^2=0.9226) between *ET* and grain yield among the three soil types under different water deficit levels. Similarly, Figure 3.2.3 also shows the best linear relation (R^2=0.9876) of grain yield and *LAI* among the three soil types under different water deficit levels.

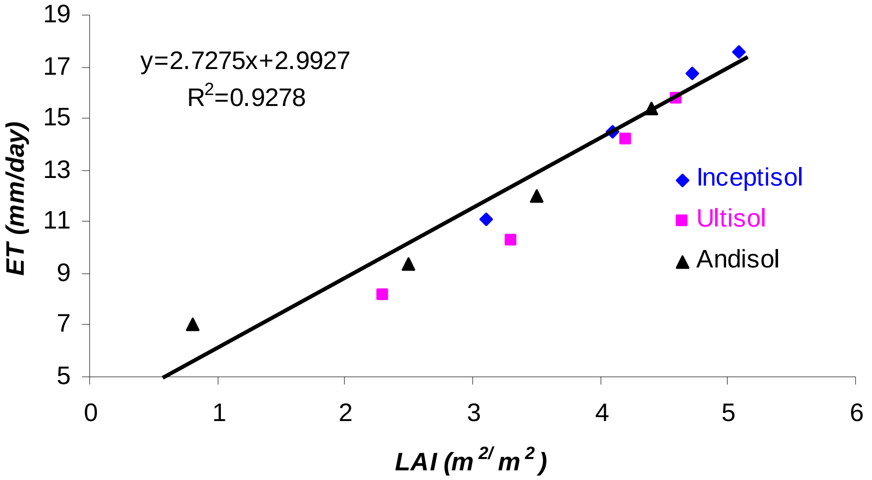


Figure 3.2.1: Relationship between *ET* (mm/day) and *LAI* (m^2/m^2) of soybean under the three soil types in response to water deficit levels.

Table 3.2.1: The effect of water deficit levels on *ET*, *LAI*, *SCMR*, leaf nitrogen, grain nitrogen and grain yield under the three soil types

Soil Types	Water Deficit Level (%)	*ET* (mm/day)	*LAI* (m^2/m^2)	*SCMR*	Leaf nitrogen (%)	Grain nitrogen (%)	Grain yield (g/pot)
Inceptisol	D_1 (0- 25)	17.54 A a	5.1 A a	35.59 A a	1.78 A a	7.30 A a	31.8 A a
	D_2 (25- 50)	16.75 A b	4.7 A b	35.57 A a	1.75A a	7.21 A b	29.5 A b
	D_3 (50- 75)	14.49 A c	4.1 A c	35.39 A bc	1.70 A b	7.08 A c	26.7 A c
	D_4 (75-100)	11.11 A d	3.1 A d	34.69 A c	1.52 A c	6.85 A d	18.8 A d
Ultisol	D_1 (0- 25)	15.75 B a	4.6 B a	35.06 B a	1.62 B a	7.11 B a	27.4 B a
	D_2 (25- 50)	14.17 B b	4.2 B b	35.05 B a	1.63 B a	7.07 B b	25.4 B b
	D_3 (50- 75)	10.30 B c	3.3 B c	34.05 B b	1.54 B b	6.95 B c	21.4 B c
	D_4 (75-100)	8.16 B d	2.3 B d	33.43 B c	1.45 B c	6.71 B d	14.8 B d
Andisol	D_1 (0- 25)	15.42 C a	4.4 B a	34.40 C a	1.60 C a	6.98 C a	26.6 B a
	D_2 (25- 50)	12.01 C b	3.5 C b	31.48 C b	1.33 C b	6.60 C b	21.5 C b
	D_3 (50- 75)	9.39 C c	2.5 C c	30.38 C c	1.12 C c	5.59 C c	17.5 C c
	D_4 (75-100)	7.02C d	0.8 C d	25.68 C d	0.70 C d	4.31 C d	6.7 C d

Means followed by different small letters (a-d) in the same column in each soil types under different water deficit levels are significantly different according to Tukey's multiple comparison test ($p<0.05$).

Means followed by different capital letters (A-C) vertically at same water deficit level among the three soil types are significantly different according to Tukey's multiple comparison test ($p<0.05$).

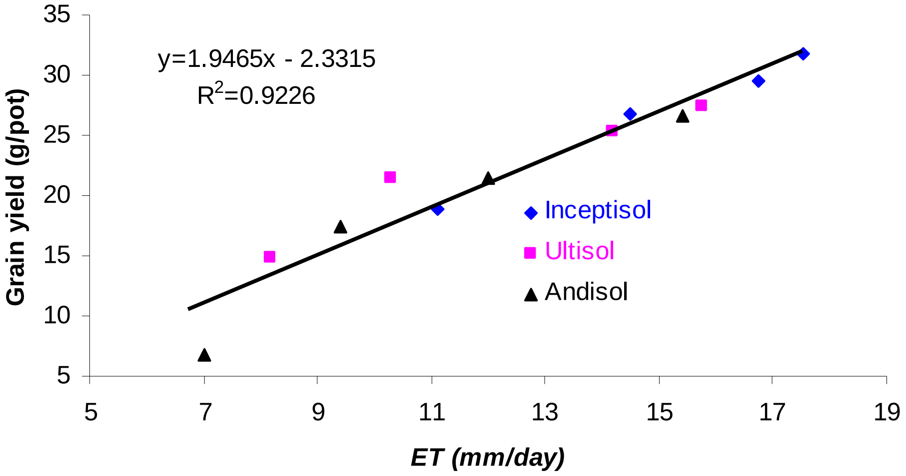


Figure 3.2.2: Relationship between *ET* (mm/day) and grain yield (g/pot) of soybean under the three soil types in response to water deficit levels.

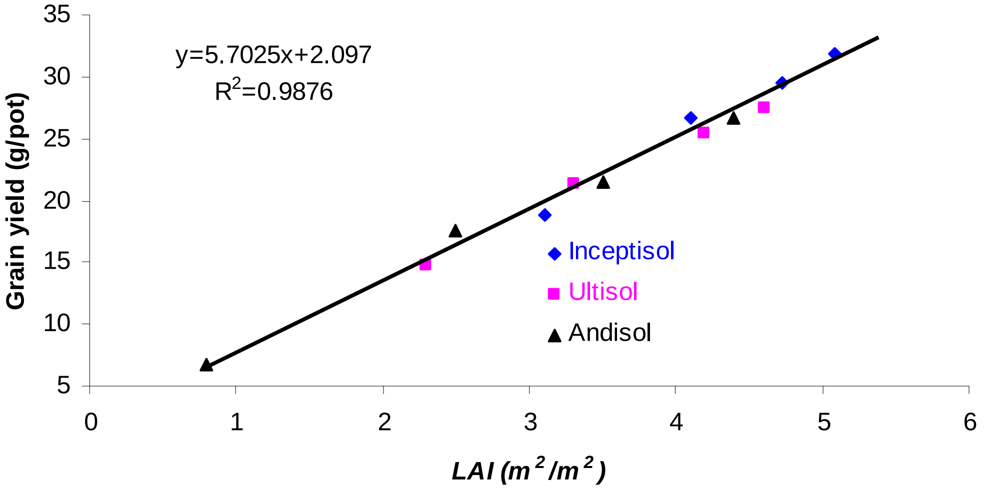


Figure 3.3.3: Relationship between *LAI* and grain yield (g/pot) of soybean under the three soil types in response to water deficit levels.

The reduction in *ET* with the decrease of *LAI* by water stress caused the decrease of soil water uptake with soluble nutrients and consequently the decrease of soybean grain yield through the reduction in photosynthesis. This result agrees with Van Wijk *et al.* (2005) who demonstrated that *LAI* is a key variable, functionally related to canopy microclimate, water interception, radiation extinction, and water and carbon exchange. Passioura (1997) found the same phenomenon that grain yield is a function of the amount of evapotranspiration. Setiyono *et al*. (2008) also demonstrated that transpiration is directly controlled by *LAI*.

3.2.2. Leaf Area Index (*LAI*) contributing to SCMR and leaf N status

The *LAI* significantly correlated with both SCMR (Figure 3.2.4) and leaf N status (Figure 3.2.5) under the three soil types in response to water deficit levels. These relationships indicated that decreasing *LAI* with the increase of water deficit levels resulted in decrease of both SCMR and leaf N status with significant differences among the three soil types under different water deficit levels. The highest *LAI*, SCMR and leaf N status was found in Inceptisol as compared to the other two soil types under all the water deficit levels.

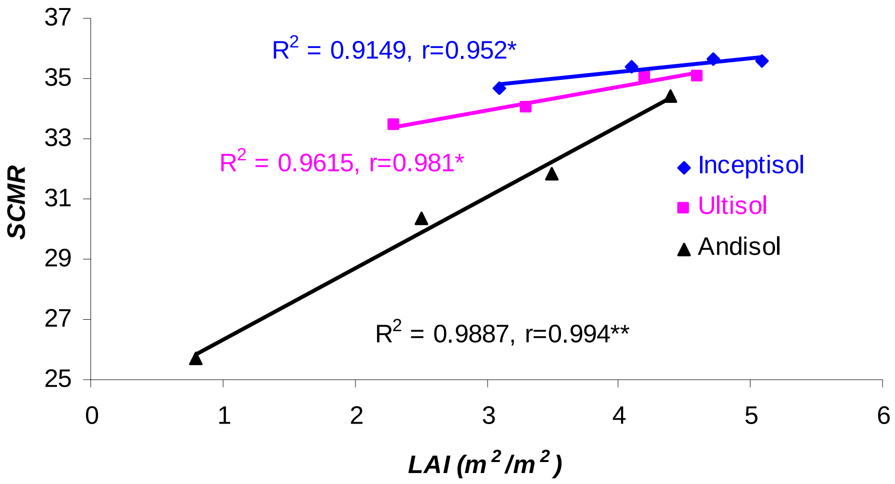


Figure 3.2.4: Relationship between *LAI* and *SCMR* of soybean under the three soil types in response to water deficit levels. *significant at $p<0.05$ and ** significant at $p<0.01$

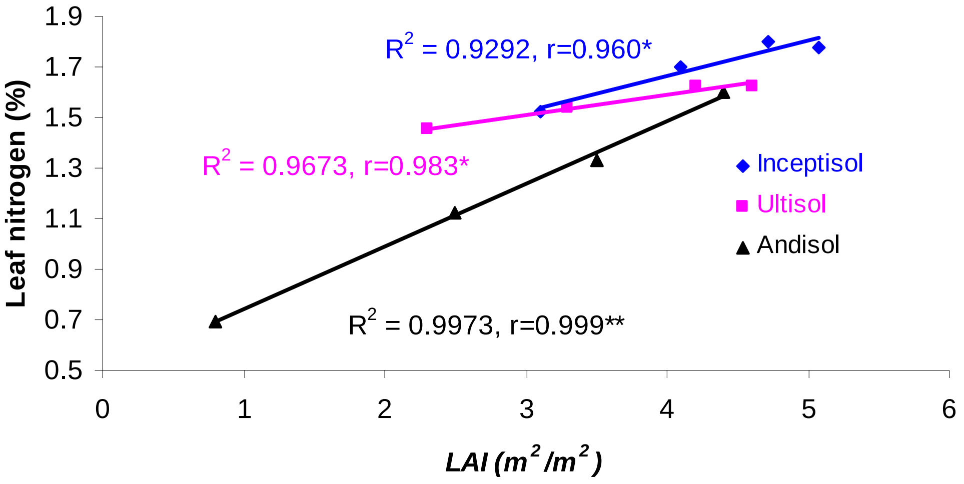


Figure 3.2.5: Relationship between *LAI* and leaf nitrogen (%) of soybean under the three soil types in response to water deficit levels. *significant at $p<0.05$ and ** significant at $p<0.01$.

The fastest decline of SMCR and leaf N status with decreasing *LAI* was found in Andisol as compared to the other two soil types in response to water deficit levels. It indicates that Andisol is the most sensitive to N assimilation under water stress conditions as compared to the other two soil types. This is mainly due to the coarse textured nature of Andisol (Table 1.1). Thompson *et al.* (1996) observed strong correlations of chlorophyll content with SPAD readings and *LAI* in soybean. They demonstrated that soybean with large leaf area had a greater potential to contribute more photosynthate to the seeds. Rate of photosynthesis and transpiration declined in soybean because of increasing water stress. Leaf area index and activity per unit leaf area are components of field photosynthetic performance (De Costa and Shanmugathasan, 2002).

3.2.3 SCMR and nitrogen status of soybean

The SCMR and leaf N status in both Inceptisol and Ultisol slightly decreased initially (non-significant) from D_1 to D_2 and then significantly declined from D_2 to D_4 water deficit levels (Table 3.2.1). In Andisol, both SCMR and leaf N status showed significant decline with the increase of water deficit levels. In addition, N status in soybean grain within each soil type significantly decreased with the increasing water deficit levels imposed from D_1 to D_4. Inceptisol had the highest SCMR and nitrogen status of soybean, followed by Ultisol and then Andisol under all water deficit levels (Table 3.2.1). Regression analysis (Figure 3.2.6) shows that SCMR correlated linearly (R^2=0.9744) with leaf N status among the three soil types in response to water deficit levels. Similarly, Figure 3.2.7 shows that leaf N status also correlated linearly (R^2=0.9534) with grain N status among the three soil types in response to water deficit levels.

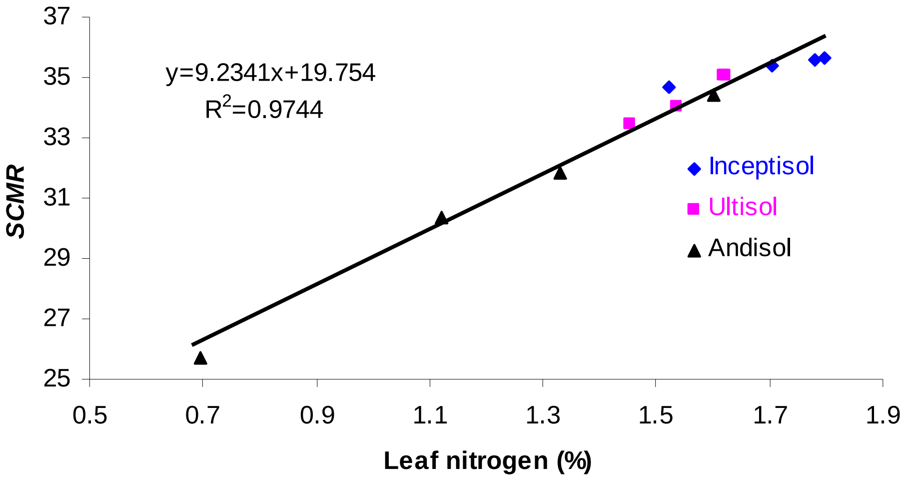


Figure 3.2.6: Relationship between *SMCR* and leaf nitrogen (%) of soybean under the three soil types in response to water deficit levels.

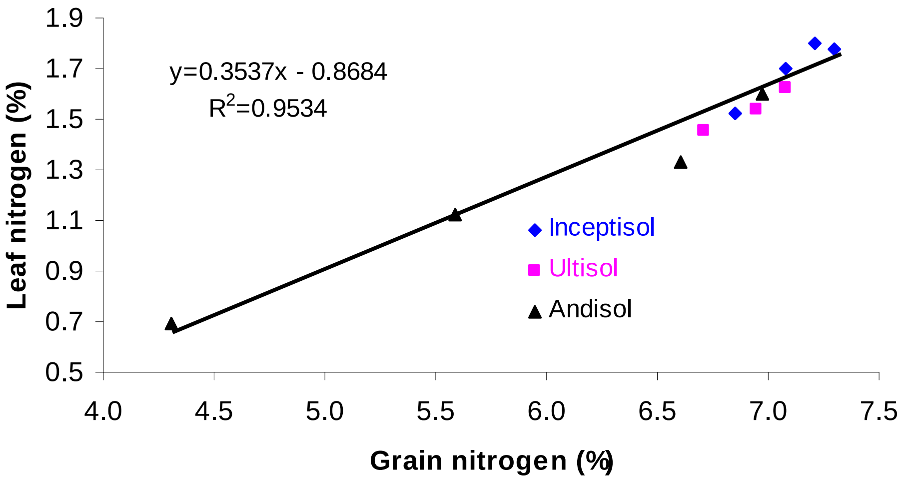


Figure 3.2.7 : Relationship between grain nitrogen (%) and leaf nitrogen (%) of soybean under the three soil types in response to water deficit levels.

Prior to using, the SPAD meter (Minolta SPAD-502 meter, Tokyo, Japan) was calibrated by organic extraction and spectrophotometric analysis according to Markwell *et al.* (1995). Based on our result, the SPAD meter can provide a quick estimation of chlorophyll in soybean leaves. Chlorophyll content is shown to be a precise indication of plant water stress (Bauerle *et al.*, 2004). Significant correlations between photosynthesis and leaf N content documented for a large number of species, including soybean exist (Evans 1989).

3.2.4 SCMR and nitrogen status of soybean contributing to grain yield

The soybean grain yield significantly correlated with SCMR (Figure 3.2.8), leaf N status (Figure 3.2.9) and grain N status (Figure 3.2.10) under the three soil types in response to water deficit levels. The highest grain yield of soybean per unit area was produced in Inceptisol, because finer-textured nature of Inceptisol retained more water that contributed to translocate more water with more nitrogen assimilated and subsequently higher grain yield than the other two soil types under the same water deficit levels. Silvius *et al.* (1977) stated that the effects of water stress on soybean yield appeared to be related to limited availability of photosynthate and nitrogen for translocation to developing seed.

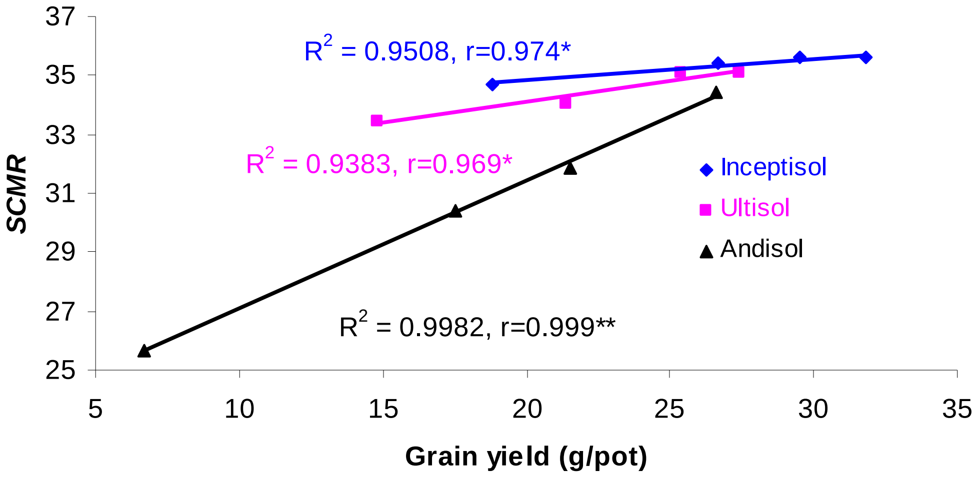


Figure 3.2.8: Relationship between grain yield (g/pot) and *SCMR* of soybean under the three soil types in response to water deficit levels. *significant at $p<0.05$ and **significant at $p<0.01$.

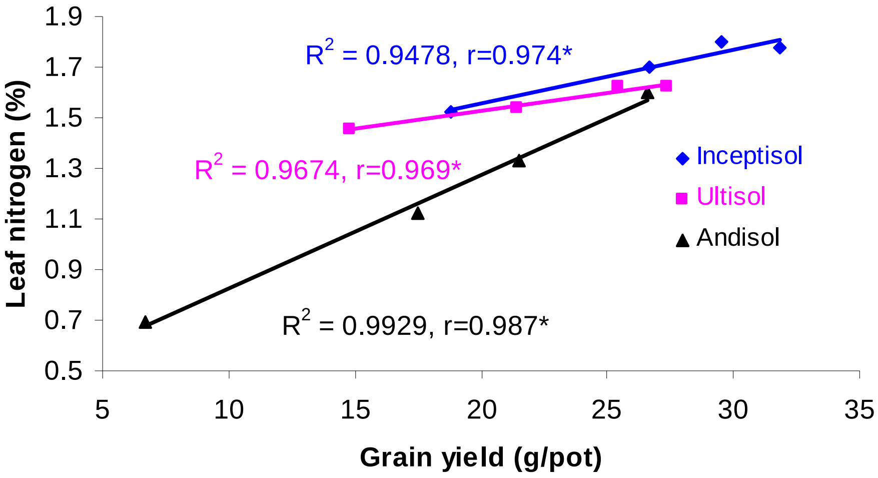


Figure 3.2.9: Relationship between grain yield (g/pot) and leaf nitrogen (%) of soybean under the three soil types in response to water deficit levels. *significant at $p<0.05$.

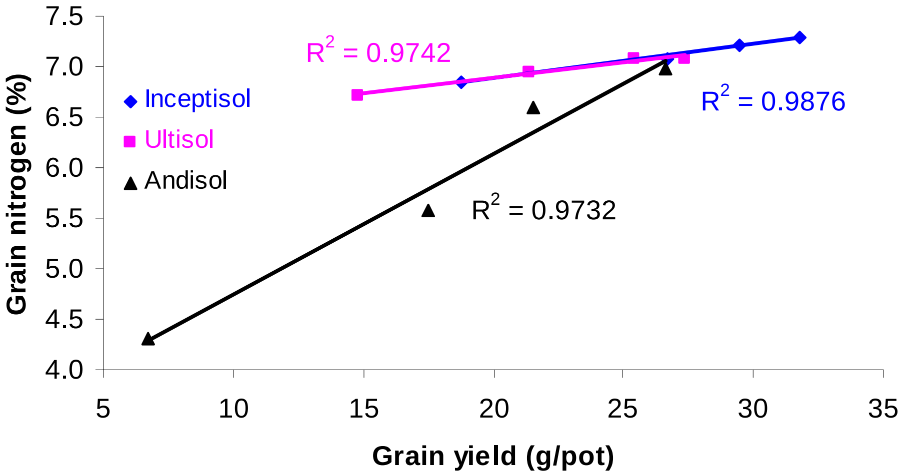


Figure 3.2.10: Relationship between grain yield (g/pot) and grain nitrogen (%) of soybean under the three soil types in response to water deficit levels. *significant at $p<0.05$.

3.3 The Effect of Deficit Irrigation on Root/Shoot ratio, Water Use Efficiency and Yield Efficiency of Soybean

3.3.1 Crop water requirement (*CWR*) and water stress coefficients (K_s)

The evapatranspiration (*ET*, mm/day) decreased with the increasing water deficit levels imposed (Figure 3.3.1). The crop water requirement (*CWR*, g/plant) of soybean at the different growth stages significantly decreased with increasing water deficit level (Table 3.3.1). Similarly, leaf area index (*LAI*, m^2/m^2) also significantly decreased at the different growth stages with increasing water deficit level. Figure 3.3.2 shows that *CWR* significantly correlated with *LAI* at different growth stage in response to the water deficit level. This result agrees with Van Wijk et al. (2005) who demonstrated that *LAI* is a key variable, functionally related to canopy microclimate, water interception, radiation extinction, and water and carbon exchange. Setiyono et al. (2008) also demonstrated that transpiration is directly controlled by *LAI*. In the present study, the highest correlation was observed at seed growth stage (77 DAS) compared to the flowering and maturity stages, because *LAI* reaches maximum at this time.

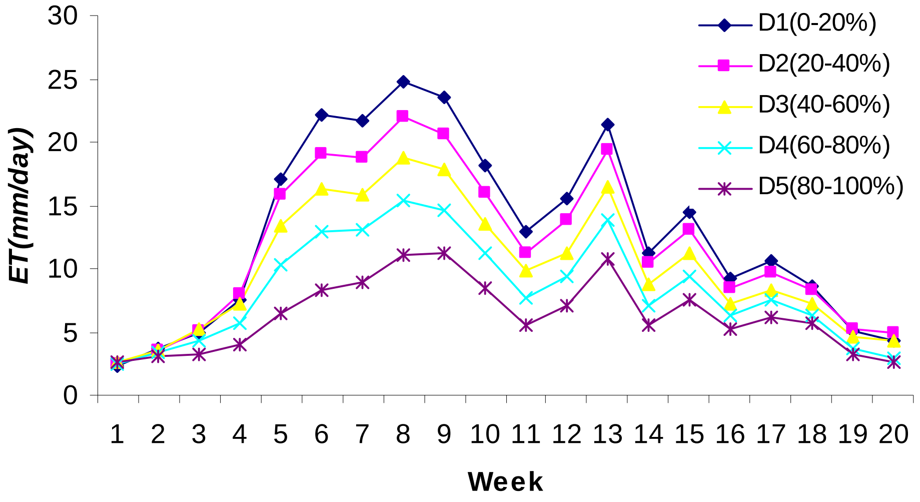


Figure 3.3.1: Evapotranspiration (mm/day) of soybean in Inceptisol.

Table 3.3.1: The effect of water deficit level on crop water requirement (*CWR*), leaf area index (*LAI*), root/shoot ratio, total dry biomass (*TDB*), water stress coefficient *(Ks)* and water use efficiency (*WUE*) of soybean under different growth stage

Growth stage (DAS)	Water deficit level (%)	*CWR* (g/plant)	*LAI* (m^2/m^2)	Shoot dry weight (g/plant)	Root dry weight (g/plant)	Root/ Shoot ratio (g/g)	*TDB* (g/plant)	*Ks*	*WUE* (g/g)
		①	②	③	④	⑤=④/③	⑥＝③+④	⑦	⑧=⑥/①
Flowering (49)	D_1	24183 a	5.8 a	48.93 a	12.70 a	0.2595 a	61.63 a	1 a	0.00255 a
	D_2	20220 b	5.6 b	40.70 b	12.07 b	0.2965 a	52.77 b	0.84 b	0.00261 a
	D_3	17754 c	4.8 c	36.50 c	11.37 c	0.3114 a	47.87 c	0.73 c	0.00270 a
	D_4	14080 d	4.5 d	29.47 d	9.60 d	0.3258 a	39.07 d	0.58 d	0.00277 a
	D_5	12867 e	3.7 e	25.30 e	5.97 e	0.2360 b	31.27 e	0.53 e	0.00243 b
Seed growth (77)	D_1	50475 a	6.7 a	87.87 a	16.87 a	0.19196 a	104.73 a	1 a	0.00207 a
	D_2	46270 b	6.5 b	81.60 b	16.57 a	0.20302 a	98.17 a	0.92 b	0.00212 a
	D_3	39039 c	5.9 c	71.00 c	14.97 b	0.21080 a	85.97 b	0.77 c	0.00220 a
	D_4	31759 d	5.5 d	59.33 d	13.60 c	0.22921 a	72.93 c	0.63 d	0.00230 a
	D_5	25608 e	4.5 e	41.37 e	7.10 d	0.17164 a	48.47 d	0.51 e	0.00189 a
Maturity (140)	D_1	82130 a	6.2 a	117.10 a	19.80 a	0.1691 b	136.90 a	1 a	0.00167 a
	D_2	74719 b	6.0 b	113.83 b	19.53 a	0.1716 b	133.37 a	0.91 b	0.00178 a
	D_3	64460 c	5.7 c	109.83 c	19.50 a	0.1775 a	129.33 b	0.78 c	0.00201 a
	D_4	53155 d	5.3 d	95.50 d	17.53 b	0.1836 a	113.03 c	0.65 d	0.00213 a
	D_5	40205 e	3.9 e	67.50 e	11.80 c	0.1748 b	79.30 d	0.49 e	0.00197 a

Means followed by different small letters (a-e) in the same column in each growth stage under different water deficit levels are significantly different according to Tukey’s multiple comparison test ($p<0.05$).

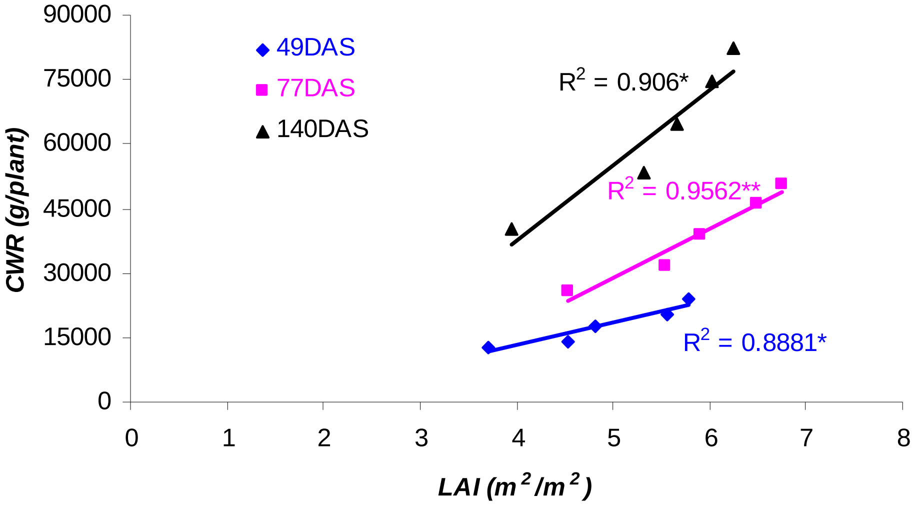


Figure 3.3.2: Effects of water stress on the relationship between leaf area index *(LAI)* and crop water requirement *(CWR)*.

According to Allen et al. (1998), evapotranspiration under water stress condition is referred to as the adjustment evapotranspiration (ET_{cadj}, mm/d) which can be calculated by the following equation.

$$ET_{cadj}=K_s\,ET_c \qquad (1)$$

where ET_c (mm/d) is the crop evapotranspiration under standard condition, K_s is water stress coefficient (no dimension).

The value of K_s is important for estimating ET_{cadj}, and can be used for deficit irrigation scheduling. K_s describe the effect of water stress on crop transpiration (Allen et al. 1998). Assuming that the evapotranspiration at D_1(0-20%) occurred under the ideal condition for plant growth in which the soil water content is near the field capacity, the actual evapotranspiration (ET_a) at D_1 is crop evapotranspiration (ET_c), which means the evapotranspiration of plant under standard conditions (Allen et al., 1998). Water stress coefficient (K_s) is calculated as the ratio between the actual evapotranspiration (ET_a) at each water deficit level and the crop evapotrarspiration (ET_c). The ratio of water depletion to the total available water in the root zone, referred to as "*p*", is an indicator of the water deficit level. For example, the average value of "*p*" under the water management of D_2 (25-50%) treatment is calculated as "*p*" = (0.20+0.40)/2=0.30.

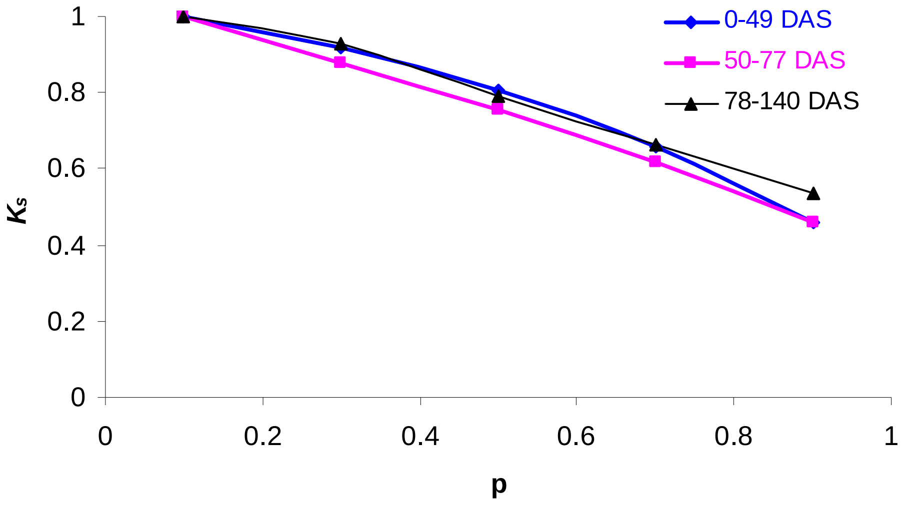


Figure 3.3.3: Effects of available water deficit (p) on water stress coefficient (K_s).

The variation of K_s values of soybean is displayed in Table 3.3.1. Water deficit level had significant effects on water stress coefficient (K_s). The variation of K_s values of soybean depends on the growth stages and the water deficit level. Figure 3.3.3 shows that the K_s values decreased linearly with the increase of water deficit level "*p*". The fastest decline of K_s value was at the seed growth stage (77 DAS) which indicates the most sensitive period to water deficit. Our result agrees with Doorenboss and Kassam (1979) who stated that, water requirement is higher during emergence, flowering and early yield formation than early (vegetative, after establishment) and late growth periods (ripening).

3.3.2 Total dry biomass (*TDB*) and water use efficiency (*WUE*)

The shoot dry weight and root dry weight as well as total dry biomass (*TDB*, g/plant) at each growth stage significantly decreased with increasing water deficit level (Table 3.3.1). However, the root:shoot ratio increased up to the D_4 and thereafter, decreased to the D_5 treatment with increasing water deficit level (Table 3.3.1). Under water stress conditions, water can be easily lost by evaporation from the surface layer of soil. Therefore, soybean root profile is characterized by a low amount of roots in the dry surface layer and a maximum root proliferation in the deeper and wetter soil layer. On the other hand, shoot growth might be restricted due to the restriction of cell division and enlargement under water stress conditions. Our results agree with Nicholas (1998) who stated that the root:shoot ratio increases under water-stress conditions to facilitate water absorption. The root growth decline was greater in the top soil than deeper soil, because water uptake per unit root length generally increased with depth in the soil.

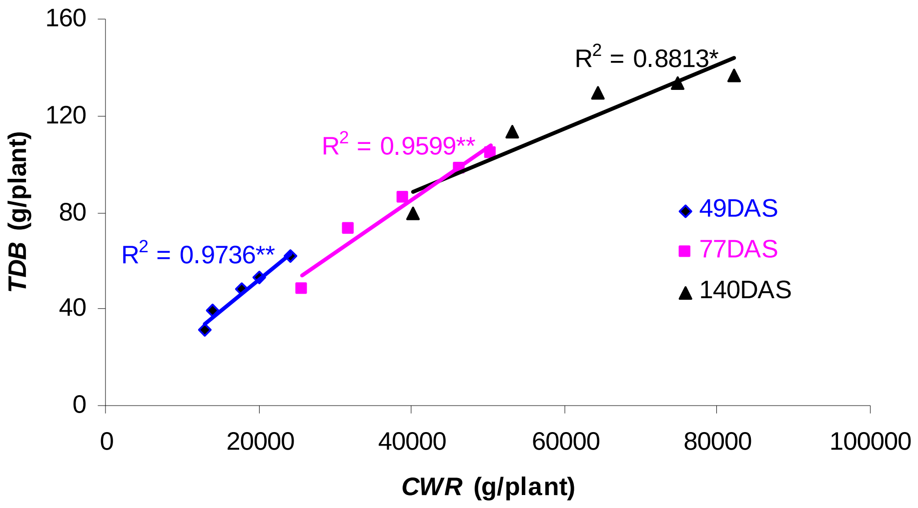


Figure 3.3.4: Effects of water stress on the relationship between total dry biomass *(TDB)* and crop water requirement *(CWR)*.

Water deficit had significant effects on total dry matter accumulation (*TDB*) (Table 3.3.1). Water deficit reduced final dry matter by an average of 42% ($P<0.05$) at D_5 treatment compared to the full irrigation (D_1). The *TDB* at different growth stage significantly correlated with *CWR* under the water deficit level (Figure 3.3.4). Our study indicates that the decrease in total dry biomass was due to the considerable reduction in plant growth and canopy structure caused by the water stress conditions. This phenomenon agrees with Hong-Bo Shao et al. (2008) who found that the biomass of soybean plant was reduced by the water stress imposed.

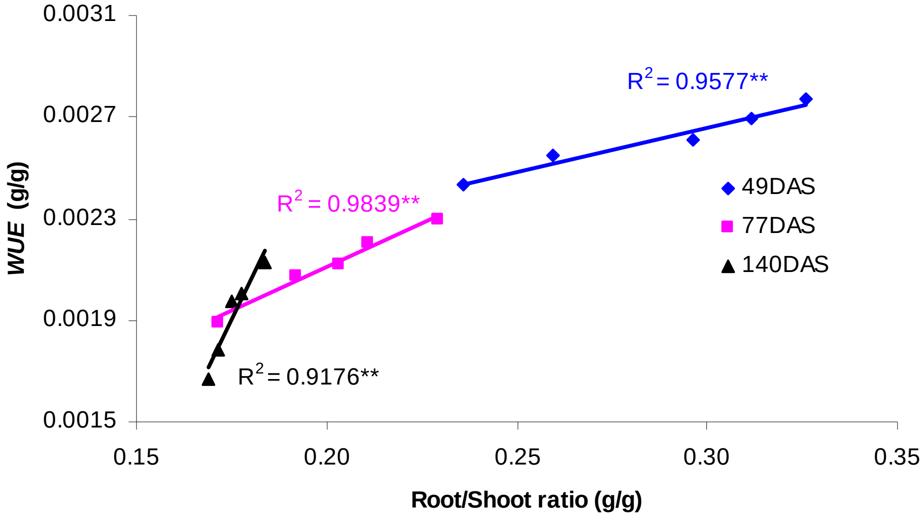


Figure 3.3.5: Effects of water stress on the relationship between root/shoot ratio and water use efficiency *(WUE)*.

Water use efficiency (*WUE*, g/g) is defined as the ratio of total dry biomass (*TDB*, g/plant) to the crop water requirement (*CWR*, g/plant). The *WUE* value increased with the increase of water deficit level, except water deficit level in D_5 (Table 3.3.1). In addition, *WUE* showed significant positive correlation at different growth stage with root/shoot ratio in response to increasing water deficit level (Figure 3.3.5). In fact, when evaluated over the entire growing season, the stressed plants have higher water use efficiencies than the well-watered plants. Our findings are in agreement with those of Burriro et al. (2002) who reported that *WUE* increased with the increase of soil moisture stress.

3.3.3 Grain yield and yield efficiency (*YE*)

The grain yield declined with the increase of water deficit levels (Table 3.3.2). There was no significant difference between the decreasing trend of D_1 and D_2 treatments but significantly decreased from D_2 to D5 treatment. It was found from the study that, the grain yield numerically lowered by 1, 11, 21 and 47 % in D_2, D_3, D_4 and D_5 water deficit levels, respectively, as compared to the D_1 (full irrigation).

Table 3.3.2: Effects of water deficit level on grain yield (Y), crop water requirement (CWR), yield efficiency (YE), and yield response factor (K_y)

Water deficit (%)	Y (g/plant) ①	CWR (g/plant) ②	YE (g/g) ③=①/②	1-Y_a/Y_m ④	1-ET_a/ET_m ⑤	K_y ⑥=④/⑤
D_1(0-20)	17.7 a	82130 a	0.000216 b	0	0	-
D_2(20-40)	17.5 a	74719 b	0.000234 b	0.01 a	0.09 a	0.13 a
D_3(40-60)	15.8 b	64460 c	0.000245 ab	0.11 b	0.22 b	0.50 b
D_4(60-80)	14.0 c	53155 d	0.000263 a	0.21 c	0.35 c	0.59 c
D_5(80-100)	9.4 d	40205 e	0.000234 b	0.47 d	0.51 d	0.92 d

Y_a: actual yield, Y_m: maximum yield, ET_a: actual evapotranspiration, and ET_m; maximum evapotranspiration

Means followed by different small letters (a-e) in the same column under different water deficit levels are significantly different according to Tukey’s multiple comparison test ($p<0.05$).

The grain yield of soybean was strongly influenced by *CWR* (Figure 3.3.6), *LAI* (Figure 3.3.7), and *TDB* (Figure 3.3.8) at maturity stage (140 DAS). These results indicated that the reduction in *CWR* as well as *LAI* and *TDB* by water stress caused the decrease of soil water uptake and consequently the decrease of soybean grain yield through reduction in photosynthesis. Passioura (1997) found the same phenomenon that grain yield is a function of the amount of evapotranspiration.

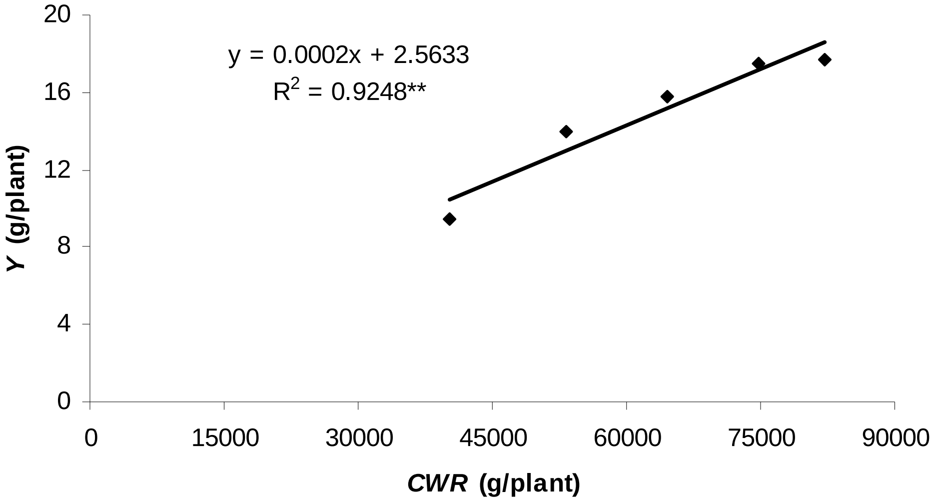


Figure 3.3.6: Effects of water stress on the relationship between crop water requirement *(CWR)* and grain yield *(Y)*.

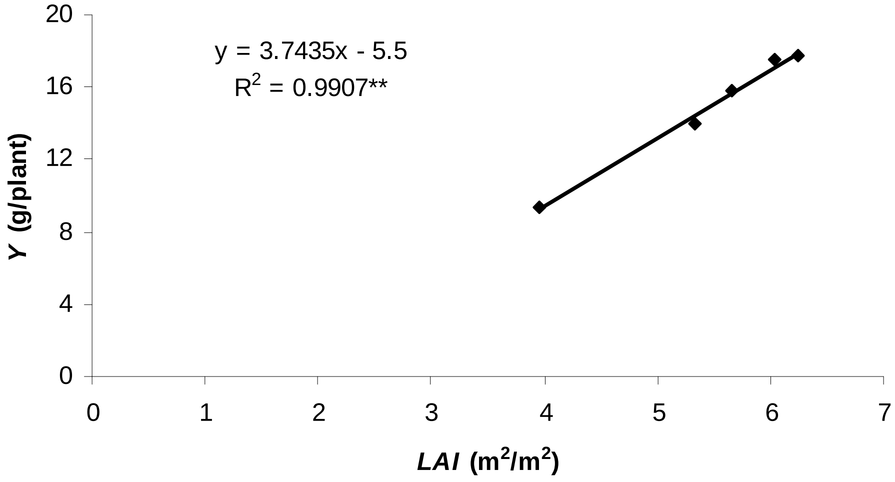


Figure 3.3.7: Effects of water stress on the relationship between leaf area index *(LAI)* and grain yield *(Y)*.

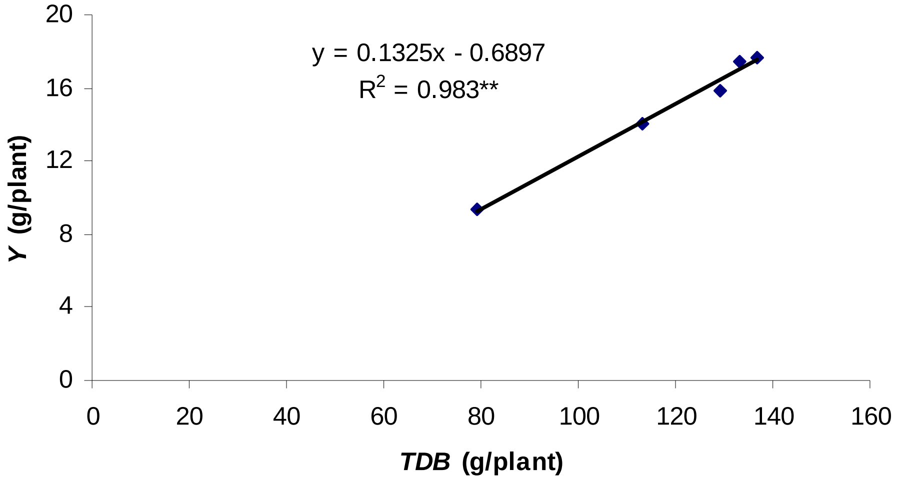


Figure 3.3.8: Effects of water stress on the relationship between biomass *(TDB)* and grain yield *(Y)*.

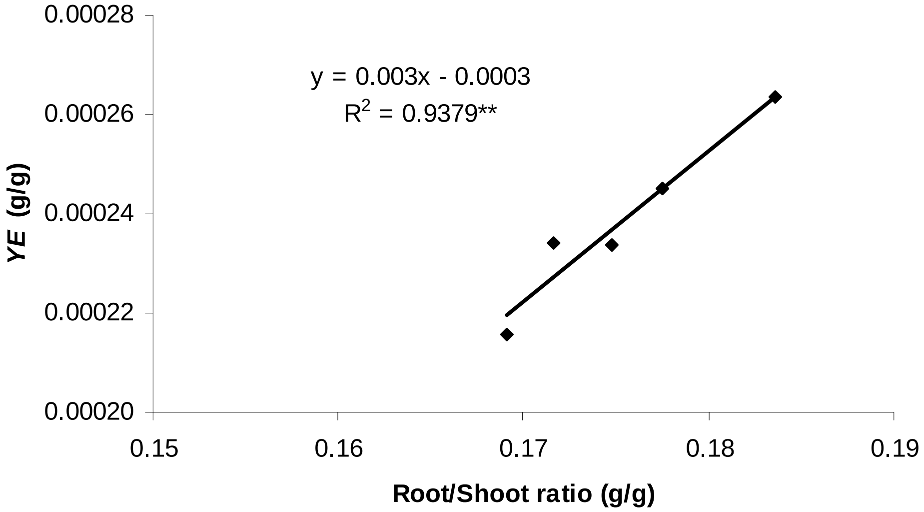


Figure 3.3.9: Effects of water stress on the relationship between root/shoot ratio and yield efficiency *(YE)*.

Yield efficiency (*YE*, g/g), the ratio of grain yield (*Y*, g/plant) to crop water requirement (*CWR*, g/plant) is a term used to assess how efficiently a crop uses water. Using less water to produce more grain yield is important in saving water. The *YE* value increased with the increase of water deficit level from D_1 to D_4 and thereafter decreased up to the D_5 treatment (Table 3.3.2). The significant positive relationship between *YE* and root/shoot ratio at maturity stage (140 DAS) showed that the *YE* increased with the increase of root/shoot ratio in response to increasing water deficit levels up to the D_4 treatment and thereafter decreased to the D_5 treatment in response to increasing water deficit levels (Figure 3.3.9). Our study indicates that, the soybean crop utilize the least irrigation water to produce more grain yield at D_4 water deficit level. Therefore, the highest *YE* were recorded at D_4 water deficit level compared to the full irrigation (D_1).

3.3.4 Yield response factor (K_y)

The effect of water stress on yield is quantified by relating the relative yield decrease to the relative evapotranspiration deficit through an empirically derived yield response factor (K_y) (Doorenboss and Kassam, 1979).

$$1-\frac{Y_a}{Y_m}=K_y\times(1-\frac{ET_a}{ET_m}) \qquad (2)$$

where 1-Y_a/Y_m: relative yield decrease, Y_a: actual yield, Y_m: maximum yield (under no stress condition), 1-ET_a/ET_m: relative evapotranspiration decrease, K_y: yield response factor, ET_a: actual evapotranspiration, and ET_m: maximum evapotranspiration

Under conditions of limited water distributed equally over the total growing season, involving crops with different K_y values, the crop with higher K_y value will suffer a greater yield loss than the crop with a lower K_y value (Moutonnet, 2000). According to a report by Doorenboss and Kassam (1979), the K_y of soybean under water deficit for the whole growing period was found to be 0.85.

Table 3.3.2 shows the values of the yield response factor (K_y) calculated using equation (2). The K_y values obtained for the D_2, D_3, D_4 and D_5 treatments were determined throughout the growing period were 0.13, 0.50, 0.59 and 0.92, respectively; with an average value of 0.53.

The relationship between relative yield decrease (1-Y_a/Y_m) and relative evapotranspiration deficit (1-ET_a/ET_m) is shown in Figure 3.3.10. It shows that, the relation between the relative yield loss (1-Y_a/Y_m) and relative water deficit (1-ET_a/ET_m) for water deficits lower than 60-80% of *TAW* is almost linear with a mean K_y value of 0.41. These results agree with the experiment by Doorenboss and Kassam (1979) who showed that the relationship between relative yield (Y_a/Y_m) and relative evapotranspiration (ET_a/ET_m) was linear and valid for water deficit of up to about 50 % or 1- ET_a/ET_m=0.5.

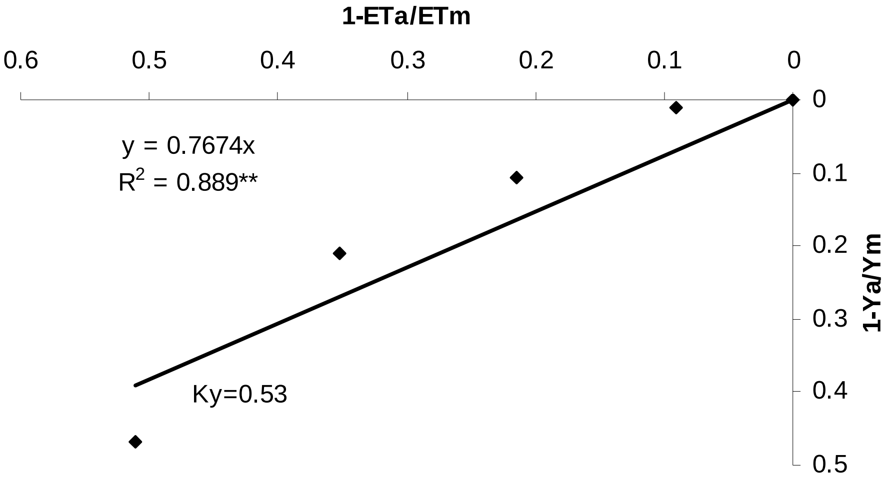


Figure 3.3.10: Relationship between relative yield decreased (1-*Ya*/*Ym*) and relative evapotranspiration deficit (1- *ETa*/*ETm*) in Inceptisol.

3.3.5 Optimum deficit irrigation

The highest values of *YE* was attained by the deficit irrigation which maintained the available water deficit at 60-80% of *TAW* (D_4) with water stress coefficient (K_s) of 0.65 and yield response factor (K_y) of 0.59. The value of *YE* at water deficit level D_4 was 1.5 times as much as under the full irrigation (D_1). It was also seen that the 60-80% (D_4) deficit irrigation reduced 21% of the grain yield per unit area, and could conserve 18% of irrigation water to produce the same grain yield compared to full irrigation (D_1).

3.4 Effects of Soil Water Stress on Nodulation, Leaf Nitrogen Accumulation and Grain Yield at Three Different Growth Stages of Soybean

3.4.1 Grain yield and leaf nitrogen accumulation under different water deficit levels

The grain yield decreased with increasing water deficit levels (Figure 3.4.1). Significant differences were observed in decreasing grain yield from $D_{3,}$ to D_5 trend but not in D_1 and D_2. The percentage reduction in grain yield compared to $D_{1,}$ was 1 for D_2, 12 for D_3, 21 for D_4, and 47 % for D_5.

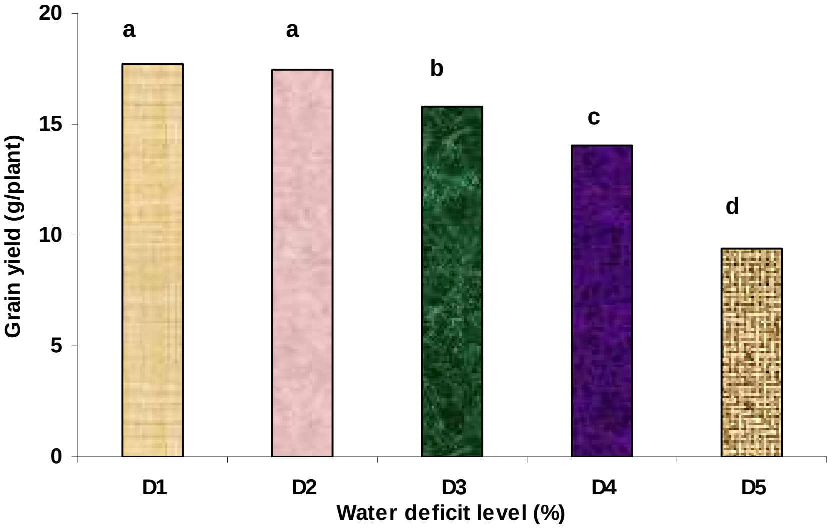


Figure 3.4.1: The effect of water deficit levels on grain yield of soybean

Means followed by different small letters (a-c) in the column under different water deficit levels are significantly different according to Tukey's multiple comparison test (p<0.05).

Leaf N accumulation was the highest in D_2 treatment, but decreased up to the D_5 at both flowering and seed growth stages. At the maturity stage, leaf N accumulation increased up to the D_3, and then decreased from D_3 to $D_{5.}$ Irrespective of the water regime treatment, leaf N accumulation was the highest at the flowering stage and the lowest at the maturity stage (Figure 3.4.2). The highest leaf N accumulation in D_2 treatment at flowering and seed growth stages indicated that irrigation scheduling of 20-40 % water deficit of *TAW* might have provided an adequate soil moisture condition that is required for establishing an efficient *Rhizobium*-host association and subsequent nodule development. This result agrees with Pahalwan *et al.* (1984) who demonstrated that under uninoculated soybean plant, more leaf N accumulation were recorded under mild water stress condition. The importance of adequate soil moisture for efficient interaction of *Rhizobium* and host was also pointed out by Gallacher *et al.* (1995).

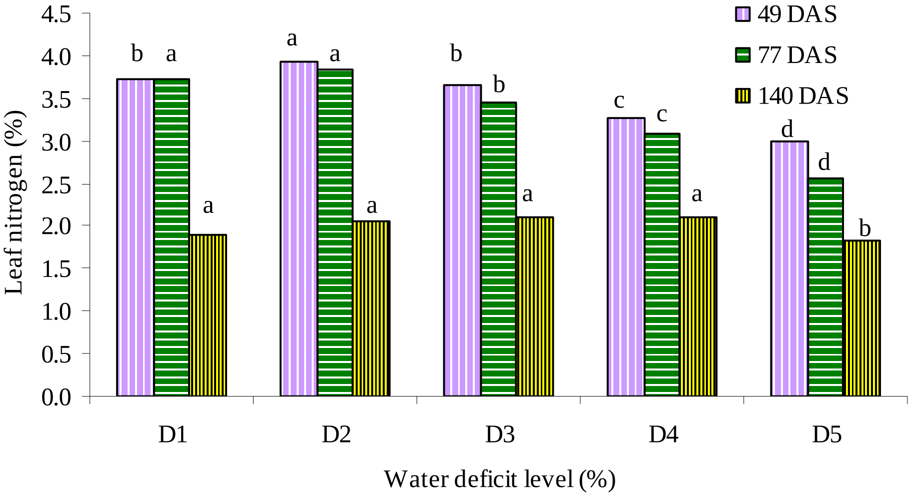


Figure 3.4.2: The effect of water deficit levels on leaf nitrogen accumulation at different growth stages of soybean.

Water deficit had positive significant effect on relationships between the leaf N accumulation and grain yield at seed growth stage (Figure 3.4.3), because physiological maturity might reach maximum at that time. Sridhara *et al.* (1995) found the same phenomenon that critically important period for fixation and assimilation of nitrogen in soybean production is during the interval between initial seed formation and the end of the linear seed-filling phase.

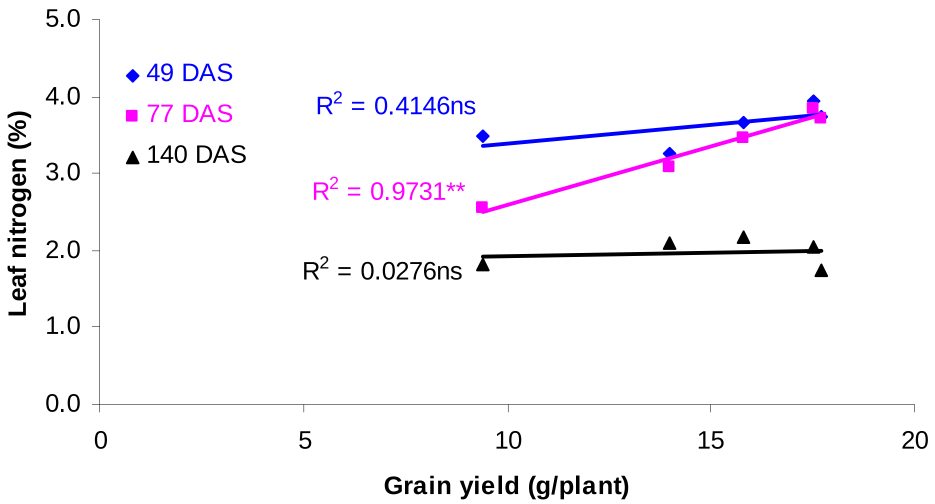


Figure 3.4.3: Plot of leaf N accumulation at different growth stages against yield (g/plant) under different water deficit levels. ns: non significant and ** significant at p<0.01.

3.4.2 Nodulation

The nodule number at ≥4.75 mm and ≤4.75 mm diameter size class as well as total and individual fresh and dry weights of nodule are shown in Table 3.4.1.

At flowering stage (49 DAS), the highest nodule number at ≥4.75 mm diameter size was recorded in D_1 treatment, while the D_2 treatment recorded the highest nodule number at ≤4.75 mm diameter. In addition, nodule number of smaller size (≤4.75 mm) was more than the larger size (≥4.75 mm). Fresh and dry nodule weights of both sizes were higher in full irrigation treatment (D_1) than the other treatments. The highest individual nodule fresh and dry weights of larger size were found in D_4 treatment, but on the contrary, the highest for the smaller size diameter class was in full irrigation treatment (D_1).

At seed growth stage (77 DAS), the highest nodule number at ≥4.75 mm size was recorded in D_3 treatment, but the highest nodule number at <4.75 mm size was in the D_2 treatment. The highest fresh and dry weights of nodules ≥4.75 mm size were in D_4 and D_3 treatment, respectively. However, D_2 treatment recorded the highest fresh and dry weights of nodules ≤4.75 mm size. The D_5 treatment recorded the highest Individual fresh and dry nodule weights for nodules $\geq$4.75 mm size. On the other hand, the highest individual fresh and dry nodule weights at $\leq$4.75 mm size were in D_1 and D_2 treatments, respectively.

At maturity stage (140 DAS), the highest nodule number for both sizes was recorded in D_2 treatment. Total and individual nodule fresh and dry weights at larger size ($\geq$4.75 mm) were the highest in D_3 treatment. On the other hand, total nodule fresh and dry weights at smaller size were the highest in full irrigation treatment (D_1). Individual nodule fresh and dry weights at smaller size (< 4.75 mm) were the highest in sever water stress conditions (D_5).

Our results indicated that water stress conditions did not always inhibit nodulation but rather sometimes enhance nodulation. In saturated soil, microbial activity is depressed by poor aeration and the limited availability of O_2 (Jinfeng *et al.*, 2000). In our full irrigation treatment (D_1), excessive water might have resulted in poor aeration, and thus reduced the number of aerobic soil microorganisms as well as nodulation. On the other hand, under mild water stress conditions (D_2), facultative anaerobic soil microorganisms might have dominated nodule production. Under D_5 treatment (which is nearer to wilting point), the severe water stress resulted in an unfavorable growth environment for the microbes, and this led to the lower nodulation. This result agrees with Sinclair *et al.* (1987) that nodulation responds to drought only when the stress was extremely severe, and that the sensitivity was distinctly different from the sensitivity of N_2 fixation to drought. Clein and Schmed (1994) also found that lower moisture contents inhibited soil microbial activity.

Table 3.4.1: The effect of water stress on nodule development parameters at different growth stages of soybean

Growth Stage (DAS)	Treatment	TNN		TNFW (mg/plant)		TNDW (mg/plant)		INFW (mg/plant)		INDW (mg/plant)	
		Nodule size class		Nodule size class		Nodule size class		Nodule size class		Nodule size class	
		≥ 4.75 mm	<4.75 mm	≥4.75 mm	<4.75 mm	≥4.75 mm	<4.75 mm	≥4.75 mm	<4.75 mm	≥4.75 mm	<4.75 mm
		①	②	③	④	⑤	⑥	⑦＝(③/①)	⑧＝(④/②)	⑨＝(⑤/①)	(10)＝(⑥/②)
49	D_1	24.0 a	39.5 a	2800 a	1580 a	870 a	530 a	117 a	40 a	36 b	13 a
	D_2	9.0 b	47.7 a	850 b	1200 ab	260 b	350 ab	94 ab	25 a	29 bc	7 a
	D_3	5.0 c	29.7 b	370 c	690 ab	90 c	220 b	74 ab	23 ab	18 bc	7 a
	D_4	4.0 c	20.0 bc	500 b	490 bc	300 b	130 c	125 a	25 ab	75 a	7 a
	D_5	7.3 b	12.3 c	830 b	430 bc	270 b	120 c	113 a	35 a	37 b	10 a
77	D_1	43.7 a	48.0 a	6000 ab	1700 a	2400 b	670 a	138 bc	36 a	55 bc	14 a
	D_2	46.0 a	55.7 a	5610 ab	1870 a	2480 ab	840 a	122 bc	34 a	54 bc	15 a
	D_3	55.0 a	42.0 ab	7980 a	1470 ab	3660 a	510 ab	145 b	35 a	67 bc	12 a
	D_4	36.3 a	33.3 bc	8020 a	680 ab	2980 a	210 b	221 ab	20 bc	82 ab	6 a
	D_5	9.3 b	20.0 c	2910 c	150 c	1060 c	30 c	312 a	8 c	113 a	1 b
140	D_1	68.7 a	51.0 a	13010 ab	2310 a	3970 a	700 a	190 ab	45 ab	58 a	14 a
	D_2	76.7 a	58.0 a	14310 ab	1630 a	4020 a	470 b	187 ab	28 bc	52 ab	8 a
	D_3	58.7 a	48.3 a	16700 a	940 b	4730 a	240 c	285 a	19 c	81 a	5 b
	D_4	39.0 bc	41.0 b	8320 c	1570 a	2610 b	510 b	213 ab	38 bc	67 a	13 a
	D_5	31.3 c	38.7 b	6150 c	2300 a	170 c	680 a	196 ab	59 a	54 ab	18 a

TNN : total nodule number, TNFW: total nodule fresh weight, TNDW: total nodule oven dry weight, INFW: individual nodule fresh weight, INDW: individual nodule oven dry weight at ≥4.75 mm diameter and <4.75 mm diameter size classes

Means followed by different small letters (a-d) in the same column in each growth stage under different water deficit levels are significantly different according to Tukey’s multiple comparison test ($p<0.05$).

3.4.3 Relationship among the nodulation, leaf N accumulation, and grain yield at different growth stages

Correlation coefficients of each nodule parameter with leaf N accumulation and grain yield are shown in Table 3 and Table 4.

There was a weak correlation and non-significant relationships among the nodulation, leaf N accumulation, and grain yield at flowering stage, except total nodule number at <4.75 mm size that showed significant positive correlation (<0.05) with yield. Leaf nitrogen and grain yield showed similar trend of relationships with fresh and dry weights of total and individual nodule weight at both nodule size classes.

On the contrary, at seed growth stage (77 DAS), total nodule numbers at ≥4.75 mm size had non-significant effect on leaf N accumulation, but positive significant effect ($p<0.05$) on grain yield of soybean. On the other hand, total nodule numbers at <4.75 mm size had positive significant effect ($p<0.01$) on leaf N accumulation and grain yield of soybean. Total nodule fresh and dry weight at ≥4.75 mm size had non-significant effect on leaf N accumulation and grain yield of soybean, but nodules at <4.75 mm size had a positive significant effect on leaf N accumulation ($p<0.01$) and grain yield ($p<0.05$). Individual nodule fresh and dry weight at ≥4.75 mm size had negative significant effect, but nodules at <4.75 mm size had a positive significant effect ($p<0.01$) on leaf N accumulation and grain yield at seed growth stage.

The leaf N accumulation and grain yield at maturity stage (140 DAS) showed non-significant correlation with nodulation parameters, except total nodule fresh and dry weights at < 4.75 mm size showed significant negative correlation ($p<0.05$) with leaf N accumulation, and total nodule number at ≥4.75 mm size showed significant positive correlation ($p<0.05$) with grain yield.

Water deficit had significant effect on relationships among the nodulation, leaf N accumulation, and grain yield at seed growth stage, because physiological maturity might reach maximum at that time. Sridhara *et al.* (1995) found the same phenomenon that critically important period for fixation and assimilation of nitrogen in soybean production is during the interval between initial seed formation and the end of the linear seed-filling phase.

Significant positive relationships among the nodulation, leaf N accumulation, and grain yield at < 4.75 mm size indicates that more successful root infection at < 4.75 mm size class nodules than the ≥4.75 mm size class nodules.

Table 3.4.2: Correlation coefficient of nodulation at ≧4.75mm and ＜4.75mm diameter size class with leaf nitrogen accumulation at different growth stages of soybean

Nodule parameter	Nodule size class	Growth Stage (DAS)					
		49		77		140	
		r	p	r	p	r	p
TNN	≧4.75mm	0.423	ns	0.737	ns	0.002	ns
	＜4.75mm	0.868	ns	0.982	0.01	0.011	ns
TNFW	≧4.75mm	0.323	ns	0.444	ns	0.398	ns
	＜4.75mm	0.731	ns	0.992	0.01	-0.954	0.05
TNDW	≧4.75mm	0.155	ns	0.553	ns	0.365	ns
	＜4.75mm	0.702	ns	0.981	0.01	-0.918	0.05
INFW	≧4.75mm	-0.556	ns	-0.984	0.01	0.677	ns
	＜4.75mm	0.040	ns	0.960	0.01	-0.810	ns
INDW	≧4.75mm	-0.770	ns	-0.992	0.01	0.660	ns
	＜4.75mm	0.106	ns	0.995	0.01	-0.768	ns

r= correlation coefficient, p= probability of significance level, and ns= non significant

Table 3.4.3: Correlation coefficient of nodulation at ≧4.75mm and ＜4.75mm diameter size class with grain yield at different growth stages of soybean

Nodule parameter	Nodule size class	Growth Stage (DAS)					
		49		77		149	
		r	p	r	p	r	p
TNN	≧4.75mm	0.470	ns	0.891	0.05	0.923	0.05
	＜4.75mm	0.917	0.05	0.964	0.01	0.868	ns
TNFW	≧4.75mm	0.405	ns	0.558	ns	0.818	ns
	＜4.75mm	0.805	ns	0.964	0.01	-0.296	ns
TNDW	≧4.75mm	0.359	ns	0.632	ns	0.867	ns
	＜4.75mm	0.788	ns	0.956	0.01	-0.289	ns
INFW	≧4.75mm	-0.303	ns	-0.982	0.01	0.047	ns
	＜4.75mm	-0.141	ns	0.964	0.01	-0.661	ns
INDW	≧4.75mm	-0.255	ns	-0.999	0.01	0.120	ns
	＜4.75mm	0.013	ns	0.976	0.01	-0.644	ns

r= correlation coefficient, p= probability of significance level, and ns= non significant

4. Conclusion

4.1 Concluding Remarks on the First Objective:

The present study indicates that the decrease of *CWR* by water stress resulted in a decrease of *LAI*, *TDB* and a subsequent decrease in soybean grain yield with significant differences among the three soil types. The soybean plants in Inceptisol could absorb and transport more water-soluble nutrients from soil through the roots with a subsequently higher grain yield due to its fine-textured properties that could retain much more water than the other two soil types.

Yield efficiency (*YE*) values indicated that soil moisture and soil aeration at the water deficit level 50-75 % of *TAW* (D_3) were the most appropriate for maximizing the *YE* values in the three soil types, and the maximum values of *YE* were slightly influenced by the three soil types.

The lowest yield response factor K_y under the water stress below 50-75% of *TAW* was in Inceptisol (0.42), followed by Ultisol (0.64) and then Andisol (0.87). These results suggest that deficit irrigation in Inceptisol (clay loam) provided the most effective economic water usage among the three soil types, followed by Ultisol (sandy clay loam) and then Andisol (sandy loam) under the water deficit level lower than 50-75% of *TAW* (D_3).

4.2 Concluding Remarks on the Second Objective:

Soybean grain yield response was linear to the *ET* and *LAI* and as well to the Soil Plant Analytical Development (SPAD) chlorophyll meter reading (SCMR) and N accumulated under the three soil types in response to different water deficit levels. The highest grain yield of soybean per unit area was produced in Inceptisol, followed by Ultisol and then Andisol under the same water deficit levels.

Our studies demonstrated that the non-destructive way of SPAD chlorophyll meter reading (SCMR) during seed formation stage is the best time for prediction of adverse water stress effects on nitrogen assimilation in determining potential yielding capacity of soybean grains. These results should be useful to select the suitable soil types for deficit irrigation management practices, which ensure optimum production of soybean.

4.3 Concluding Remarks on the Third Objective:

The most unique result of our study established a strong positive correlation among the root/shoot ratio, water use efficiency (*WUE*) and yield efficiency (*YE*) of soybean under deficit irrigation management. Our present study suggests that the yield efficiency increased with the increase of water use efficiency as well as the increase of root:shoot ratio in response to increasing water deficit levels. The study showed that, the most effective economic water usage with the highest *YE* was at D_4 (60-80 % of *TAW*) water deficit. It could produce 21% lower yield per unit area, but could conserve 18% irrigated water to produce the same yield compared to the potential yield produced under the full irrigation (D_1).

4.4 Concluding Remarks on the Forth Objective:

Our studies demonstrated that the water deficit level D_2 (20-40% of *TAW*) was the best for an efficient *Rhizobium*-host association and subsequent nodule development. Water deficit had significant effect on relationships among the nodulation, leaf N accumulation, and grain yield at seed growth stage, because physiological maturity might reach maximum at that time. Based on our results, it can be concluded that successful root infection of uninoculated soybean was more pronounced in < 4.75 mm diameter size class nodule than the larger ones ($\geq$4.75 mm) under different water deficit levels.

Given the relationship of nodulation and leaf N accumulation with grain yield, it is obvious that no one single character was important for grain yield. Yield is a complex terminal outcome of growth to which there are diverse and interrelated development tracks. However, based on our results, it appears that nodulation, leaf N accumulation, and grain yield are important characters to consider during soybean cultivation under deficit irrigation practices.

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