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THESIS

PHYSIOLOGICAL RESPONSES OF SELECTED SOYBEAN  
CULTIVARS [*Glycine max* (L.) Merrill]  
UNDER DIFFERENT WATER REGIMES



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A Thesis Submitted in Partial Fulfillment of  
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Aung Moe Myo Tint 2011: Physiological Responses of Selected Soybean Cultivars [*Glycine max* (L.) Merrill] under Different Water Regimes. Doctor of Philosophy (Tropical Agriculture), Major Field: Tropical Agriculture, Interdisciplinary Graduate Program. Thesis Advisor: Associate Professor Ed Sarobol, Ph.D. 157 pages.

Soybean has a fairly wide range of adaptation involving a wide array of climates and soil, though it is mostly grown on rain-fed land. Drought is a major environmental factor determining crop yield under rain-fed conditions due to erratic rainfall. At present scenario of climate changes, it has been predicted that drought is more likely to occur. Therefore, improving drought tolerant cultivars with better water use efficiency has been concerned as a priority for drought prone area and forwarding to water saving agriculture. However; many mechanisms are underlying plant response to drought stress. The situation devoted for cost effective selection criteria for drought tolerant cultivar improvement. This study aimed at investigating on physiological responses of soybean cultivars to seek for potential cost effective selection criteria for selecting drought tolerant cultivars with a numbers of experiments, conducted in the greenhouses and in the field.

Among the soybean cultivar, SJ.4 was the most appropriate cultivar not only for cultivar improvement but also for crop production in drought prone area because its physiological properties responding to drought and better yield potential in drought stress environments. Stress tolerance index (STI) was the most appropriate yield based index for selecting drought tolerant cultivar, followed by geometric mean productivity (GMP). The specific leaf weight (SLW) trait showed significant correlation with photosynthetic parameters, fluorescence parameters, water use efficiencies parameters and number of seed yield; followed after shoot dry matter (SDM) accumulation of a given genotype. Therefore, SLW trait could be used as an alternative potential cost effective criterion for selecting productive genotypes for both favorable and drought stress environments. N<sub>2</sub> fixation is sensitive to water deficit. Nitrogenase activity (NA) of nodulated root had significantly decreased 17.73% of control since 7 DAV<sub>4</sub> (R<sub>3</sub>). Not only NA of nodulated root severely diminished, 87.10% of control at 12 DAV<sub>4</sub> (R<sub>4</sub>) under progressive soil drying and did not recovered well after re-watering but also in nodule growth (NDM). For selecting greater soybean cultivar for N<sub>2</sub> fixation tolerant under water deficit, nitrogen concentration in accumulated shoot biomass under well watered could be used as an alternative indicator. SPAD (M-502) could be used as a powerful tool for selecting not only for productive genotypes but also for N<sub>2</sub> fixation drought tolerant soybean cultivars since it had significant high correlations with those potential traits; SLW, SDM and NDM.

Drought occurrence at late reproductive stage (R<sub>4</sub>) caused greater soybean seed yield reduction (23%) than occurred at V<sub>4</sub>; just before flowering (21 %) even sufficient rain occurred during imposed drought period for 14 days which started at R<sub>4</sub>.

Student's signature

Thesis Advisor's signature

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June, 2011

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## LIST OF ABBREVIATIONS

AcN	=	Accumulated nitrogen in accumulated shoot biomass
AP	=	Apparent photosynthesis
ARA	=	Acetylene reduction assay
BNF	=	Biological nitrogen fixation
Chl	=	Chlorophyll
Chl <sub>a</sub>	=	Chlorophyll a
Chl <sub>b</sub>	=	Chlorophyll b
Chl <sub>t</sub>	=	Total Chlorophyll content
CMS	=	Cell membrane stability
DAWW	=	Days after withholding water
DAV <sub>3</sub>	=	Days after vegetative growth stage 3
DAV <sub>4</sub>	=	Days after vegetative growth stage 4
DAR <sub>4</sub>	=	Days after reproductive growth stage 4
EL	=	Electrolyte leakage
IDNW	=	Individual nodule dry weight
NA	=	Nitrogenase activity
NAR	=	Nodule dry matter accumulation rate
Nconc	=	Concentration of total N in accumulated shoot biomass
NDM	=	Nodule dry matter
Pmax	=	Saturated photosynthesis
PSI	=	Photo system I
PSII	=	Photo system II
R	=	Reproductive growth stage
RAR	=	Root dry matter accumulation rate
RM	=	Root mass
RDM	=	Root dry matter

**LIST OF ABBREVIATIONS (Continued)**

RWC	=	Relative water content
SAR	=	Shoot dry matter accumulation rate
SDM	=	Shoot dry matter
SLA	=	Specific leaf area
SLW	=	Specific leaf weight
SM%	=	Soil moisture percentage
SPAD	=	Soil Plant Analysis Development
STM%	=	Shoot moisture percentage
SYd	=	Seed yield
TNN	=	Total nodule number
V	=	Vegetative growth stage
WUE	=	Water use efficiency

# PHYSIOLOGICAL RESPONSES OF SELECTED SOYBEAN CULTIVARS [*Glycine max* (L.) Merrill] UNDER DIFFERENT WATER REGIMES

## INTRODUCTION

Soybean (*Glycine max* [L.] Merrill) is a legume that grows in tropical, subtropical and temperate climates. Soybean seed is rich in protein and oil content and providing high energy food for growing population. Varieties of by-products from soybean are used in many industries and animal husbandry across the world. Soybean has been recognized as a crop for sustainable crop production for its ability for symbiosis nitrogen fixation. Moreover, soybean is one of the most promising crops for producing bio-energy (bio-diesel) in the near future (Soy Stats, 2009).

Soybean has a fairly wide range of adaptation involving a wide array of climates and soil, though it is mostly grown on rain-fed land (Fageria, 1997). Recently, climate-change scenarios indicate an increase in aridity in many regions of the world (Petit *et al.*, 1999; IPCC, 2007). Drought is a major environmental factor determining crop yield, especially in the tropics and sub-tropics for crop production under rain-fed conditions due to erratic rainfall. At present scenario of climate changes, drought is more likely to occur and it may decrease average yields of the most important crops globally (Bray *et al.*, 2000). In the other aspect, the biggest consumer of water is agriculture, which account for around 70% of all fresh water withdrawals worldwide. With a growing population and food demand, agriculture will have to face more competition with industrial and domestic water users. Therefore, agriculture will have to use water more efficiently. Accumulated evidences suggested that cultivar improvement for drought prone area and forwarding to water saving agriculture, improving drought tolerant cultivars with better water use efficiency has been concerned as a priority. Selection for drought resistant cultivar based solely on seed yield could not assure to gain the desired physiological trait without physiological investigation for crop improvement (Subbarao *et al.*, 1995). Therefore,

it has been undertaking crop improvement with integrated approach from traditional to molecular techniques (Hammer and Jordan, 2007; Manavalan *et al.*, 2009) including physiological aspect. In addition, sensitivity of nodulation and N<sub>2</sub> fixation to soil drying has been considered as major limiting factor to improve soybean yield (Serraj *et al.*, 1999), and this may restrict nitrogen supply and yield of soybean in many environments. In soybean, nitrogen fixation differed in response to water deficit, depended on both the inheritance of given genotypes and severity of the water stress (Sinclair *et al.*, 2000). Better understanding in nitrogen fixation of particular soybean cultivars responded to water deficit could improve soybean yield for given environment (Sinclair *et al.*, 2007) and innovation of other managements (Hungria and Vargas, 2000).

Since many mechanisms are underlying in plant response to drought (Chaves and Oliveira, 2005), therefore, several physiological traits have been considered for selecting the drought tolerant cultivars. Furthermore, genotypic differences in the particular trait of soybean have been reported. Investigations on physiological responses to drought stress are laborious and time consuming. Therefore, such screening technique and criteria are devoted for selecting a drought tolerant cultivar from such a large number of genotypes. This recalls for a deeper understanding about the physiological mechanism of the crop plants response to soil water deficit (Turner, 1997). Researches in this study aimed to evaluate on drought tolerance attributions of some cultivated soybean cultivars which commonly grown in Thailand, with consideration on their physiological responses. In addition, some possible physiological traits related to drought tolerance that can be useful as cost effective criteria for selecting drought tolerance in soybean.

## OBJECTIVES

### Overall objective

To observe physiological responses of some selected soybean cultivars to different water regimes which may contribute to drought tolerant cultivar improvement and significant yield increased in soybean production.

### Specific Objectives

1. To evaluate some soybean cultivars for their drought tolerance.
2. To investigate relationships between the physiological traits concerned as cost effective criteria for selecting drought tolerant soybean cultivar and other physiological parameters.
3. To evaluate N<sub>2</sub> fixation of some soybean cultivars under different water regimes.
4. To determine soybean seed yield reduction due to effect of drought occurrence during reproductive growth.

## LITERATURE REVIEW

### 1. Crop origin and cultivation history

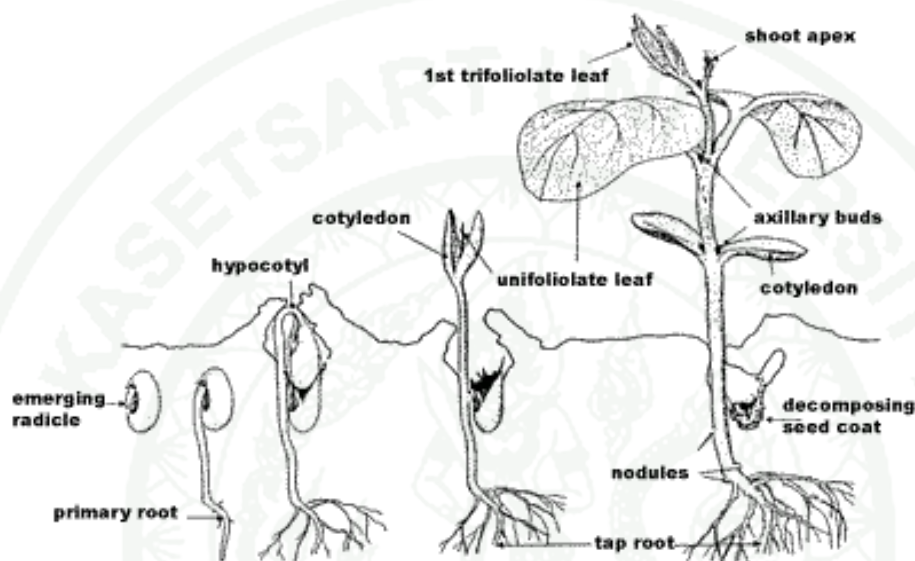
The earliest record of soybean cultivation is from China and there is general agreement that soybean plant has its origin in the northern provinces of China and Manchuria (Qiu and Chang, 2010). Soybean belonged to the family Leguminosae, sub-family Papilionoidae, and the genus *Glycine* L. The genus *Glycine* Willd. is divided into two subgenera, *Glycine* (perennials) and *Soja* (Moench) F.J. Herm. (annuals). The vining perennials species of soybean were also found in Australia, Africa and south west and east of Asia (Norman, 1978; Qiu and Chang, 2010). The cultivated species is *Glycine max* (L.) (Fageria *et al.*, 1997) Soybean was introduced in Europe in 1712 and in America in the early 1800's (Whigham, 1983) and eventually the scientific records of soybean were appeared in Western Europe and the United States (Norman, 1978). At present, soybean has been grown many countries across the world. The major soybean producers are the U.S.A., Brazil, Argentina and China (FAO, 2008).

### 2. Soybean Growth and development

Three types of growth habits can be found among soybean cultivars: determinate, semi-determinate and indeterminate. Determinate growth is characterized by the cessation of vegetative activity of the terminal bud when it becomes an inflorescence at both axillary and terminal racemes. Indeterminate genotypes continue vegetative activity throughout the flowering period (Kumudini, 2010). Semi-determinate types have in-terminate stems that terminate vegetative growth after the flowering period

Knowledge of crop phenology is useful in all aspects of crop improvement and management. Fehr *et al.* (1977) developed a system for stage soybean development that has been gained wide acceptance. The soybean is a dicotyledonous plant that

exhibits epigeal (above the surface) emergence; during germination, the cotyledons are pushed through the soil to the surface by elongating hypocotyls. Under favorable environmental conditions, seedling begins to emerge in 4 or 5 days after sowing.



**Figure 1** Germination and seedling development of soybean.

**Source:** University of Minnesota (2011)

Soybean development is characterized by two distinct growth phases. The first is the vegetative stages (V) that cover development from emergence through flowering. Plant stages are determined by classifying leaf, flower, pod, and or seed development. Staging also requires node identification beginning with the unifoliate node that has a completely unrolled. The second phase is the reproductive (R) stages from flowering through maturation (Table 1). Flowering may occur over 4-6 weeks, depending on the environment and cultivar. After fertilization of the flower, the pods develop slowly for first few days, and then the rate of development increases until the pod reaches maximum length after 15-20 days (Whigham, 1983). Soybeans has a high percentage of self-fertilization and cross-pollination is usually less than one percent as a result of androecium and gynaecium development and timing of anthesis and receptiveness of the stigma (Abernethy *et al.*, 1977).

**Table 1** Description of vegetative (V) and Reproductive (R) growth stages in soybean.

Growth Stages	Description
V <sub>1</sub>	Completely unrolled leaf at the unifoliate node
V <sub>2</sub>	Completely unrolled leaf at the first node above the unifoliate node
V <sub>3</sub>	Three nodes on the main stem beginning with the unifoliate node
V <sub>(N)</sub>	N nodes on the main stem with the unifoliate node
R <sub>1</sub>	One flower at any node
R <sub>2</sub>	Flower at node immediately below the uppermost node with a completely unrolled leaf
R <sub>3</sub>	Pod 0.5 cm long at one of the four uppermost nodes with a completely unrolled leaf
R <sub>4</sub>	2 cm Pod long at one of the four uppermost nodes with a completely unrolled leaf
R <sub>5</sub>	Seeds beginning to develop at one of the four uppermost nodes with a completely unrolled leaf
R <sub>6</sub>	Pod containing full-size green seeds at one of the four uppermost nodes with a completely unrolled leaf
R <sub>7</sub>	Pod yellowing; 50% of leaves yellow. Physiological maturity
R <sub>8</sub>	95% of pods brown. Harvest maturity

**Source:** Fehr *et al.* (1977)

### 3. Water Requirement of Soybean

Water requirement of soybean is depending on genotypes, edaphic factors, climatic factors, management and cultural practices for particular location. The total water consumption of soybean varied from 250-850 mm throughout growth period of soybean where the growing season range from 100 days at low latitude up to 190 days

in higher latitudes (Doorenbos and Pruitt, 1977; Neumaier and Nepomucen, 1992). Generally, soybean consumes 70-75 % of total water consumption during reproductive growth ( $R_1$ - $R_8$ ) while 20-25 % in vegetative growth stages ( $V_1$ - $V_n$ ). Approximately, soybean consumes about 45 % of total water requirement during pod filling stage (Neumaier and Nepomucen, 1992). Therefore, most of studies relate to water management have focused on reproductive growth stages of soybean. The most studies pointed out that from flowering to following reproductive stages is critical determining period for soybean yield (Meckel *et al.*, 1984; Desclaux *et al.*, 2000; de Costa and Shanmugathan, 2002; Brevedan and Egli, 2003).

#### **4. Water deficit affects on soybean growth and yields**

The degree of crop sensitivity to water deficit, during different development stages of a plant, is as important aspect to consider, especially in semi-tropical regions. Jones and Jones (1989) defined water stress as the lack of amount of soil water that needed for plant growth and development which affect various metabolic processes. More serious stresses that have the potential for inducing irreversible cell injury in plant are called severe stress. Water deficit due to drought may affect various physiological and morphological aspects of the soybean plant, which in turn affect on nodulation, nitrogen fixation, growth and yield. Under water deficit condition, assimilation and transport of photoassimilate (Ohashi *et al.*, 2000; 2006), leaf enlargement (Boyer, 1970), leaf area and specific leaf area development (Shin *et al.*, 2005) were delayed and resulted in retarded growth.

According to Sionit and Kramer (1977), water stress decreased total leaf area and leaf weight. Pandey *et al.* (1984) also found that increasing drought stress progressively reduced leaf area, leaf area duration (LAD), crop growth rate (CGR), and shoot dry matter. Among the processes, photosynthesis is one of potentially limiting soybean yield. Leaf area index (LAI) and activity per unit leaf area are components of field photosynthetic performance (De Costa and Shanmugathan, 2002). Thus, total photosynthesis of water-stressed plants is decreased by reductions in photosynthetic surface area caused by decreased leaf enlargement. Stomatal

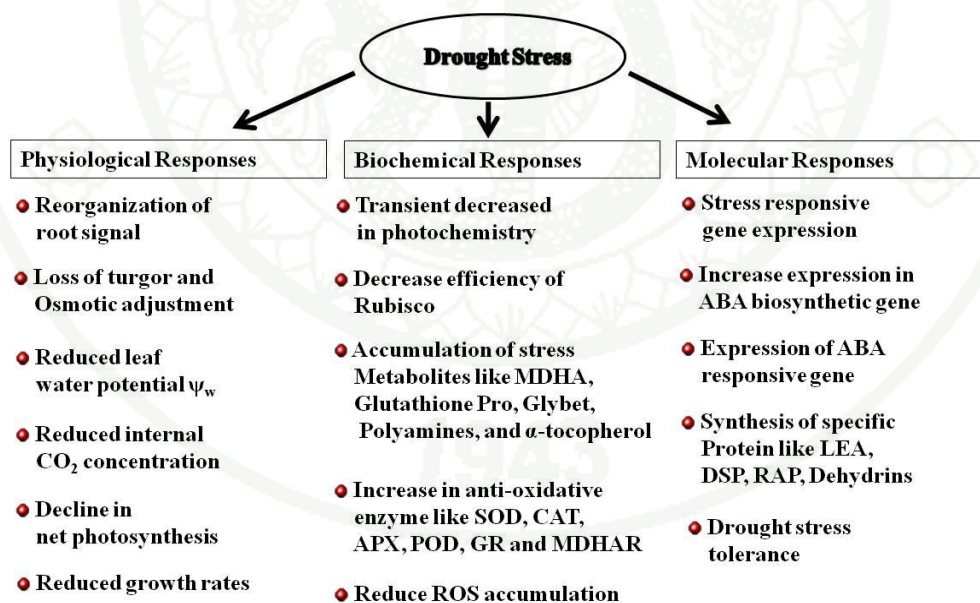
conductance, photosynthetic rates and transpiration decreased simultaneously in water-stressed soybean plants. Raper and Kramer (1987) also reported that effect of drought stress on photosynthetic rates of soybean declined rapidly with further reductions in leaf water potential to about -1.8 Mpa, and then continue to decline gradually with decreasing water potential.

Large plants with many nodes on both the main-stem and branches have more potential for pod production in soybean. Shoot size at time of flowering accounted on height of the main-stem and length of the branches, number of nodes, and total leaf area have been reported to have a significant effect on soybean yield. Drought stress affected the number of nodes per plant and pods per node, but the response varied among cultivars which interact with timing of drought stress (Kadhem *et al.*, 1985). Accumulated evidence indicates that soybean yield is more sensitive to drought stress during the early reproductive stage (flowering to early pod expansion) than other development stages (Boyer, 1983; Westgate and Peterson, 1993). Drought stress occurring during this period increases the rate of pod abortion (Liu, 2004), leads to a less number of pods per plant (Desclaux *et al.*, 2000), and ultimately decreases seed yield (Kokubun *et al.*, 2001). Brvedan and Egli (2003) reported that the most critical period of drought is from before flowering to seed-filling period.

Soybean seed yield was 23% reduced under drought condition and contribution of branch seed yield was more affected (Frederick *et al.*, 2001). The seed yield obtained from late stress relief during seed filling period was 11% lower than the early drought relief (Brevedan and Egli, 2003). As major yield components; number of pods per plant and number of seeds per pods were decreasing up to 35% and 50%, respectively, when drought was prolong (Shin *et al.*, 2005). Seed per pods, individual seed weight and vegetative dry matter were significantly reduced due to drought occurrence during seed filling period than other growth stages (Desclaux *et al.*, 2000). Individual seed size is reduced by drought, especially from branches (Frederick *et al.*, 2001). Seed protein content was increased (4.4%) while oil content was decreased (2.9%), under severe drought condition (Dornbos Jr. and Mullen, 1991).

## 5. Drought response mechanisms in the higher plants

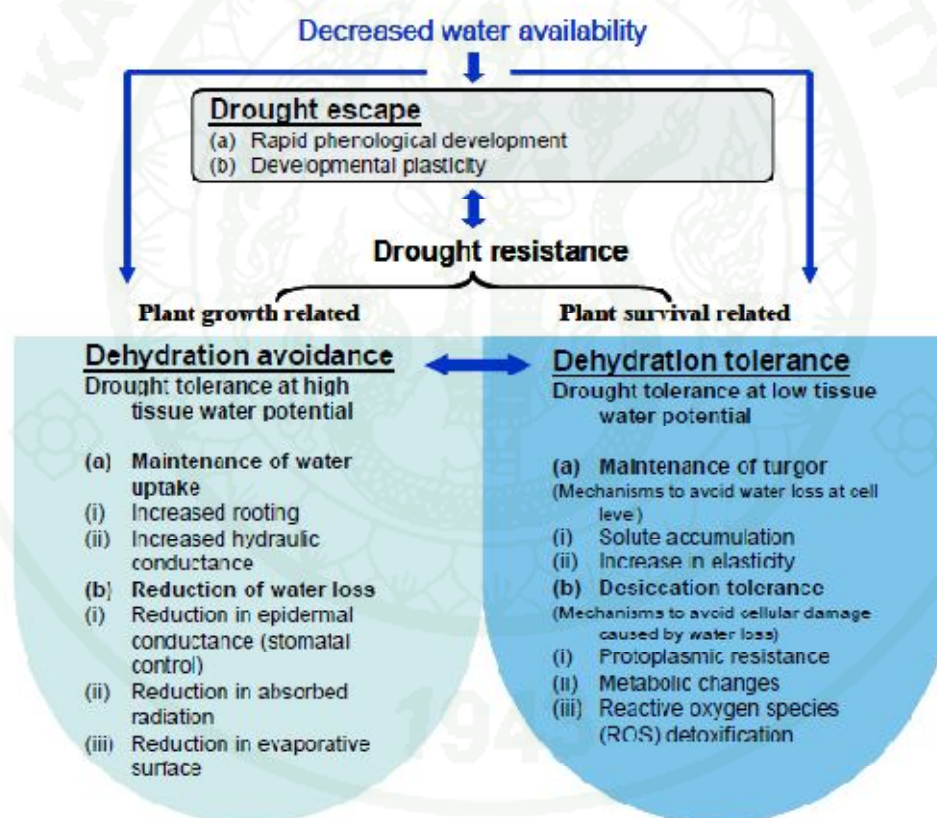
Drought stress is one of most important yield limiting factors in crop production and it could occur anytime during crop growing season. Drought can be defined as higher evaporation demand than precipitation or irrigation into soil. A drought situation can be classified as either terminal or intermittent. During terminal drought, the availability of soil water decreases progressively and this leads to severe drought stress at the later period of crop growth and development. Intermittent drought is the result of finite periods of inadequate rain or irrigation occurring at one or more intervals during the growing seasons and is not necessarily lethal. When higher plants were exposed to drought stress, plants use various mechanisms to cope with drought stress for their survival (Brays *et al.*, 2000; Baetels and Sunkar, 2005). Reddy *et al.* (2004) summarized on various responses of plants to drought stress into physiological, biochemical and molecular responses (Figure 2).



**Figure 2** Physiological, Biochemical and Molecular responses to drought stress in higher plants.

**Source :** Reddy *et al.* (2004)

In physiology aspect, various physiological mechanisms responded to drought are classified into three groups; drought escape, drought avoidance and drought tolerance (Turner *et al.*, 2001). Rehman (2009) reviewed on drought response mechanisms and organized under those three categories; drought escape, dehydration avoidance or drought tolerance, and dehydration tolerance or drought avoidance (Figure 3). Quisenberry (1982) defined drought resistance as the ability of a plant variety to produce a higher yield than another at a given limiting level of water availability.



**Figure 3** Crop plant adaptation mechanisms in response to decreased water availability.

**Source:** Rehman (2009)

Either before or under the one set of water deficit, plants have completed its life cycle termed as drought escape. Normally the life cycle is shorter and plants set some seeds instead of complete crop failure. The second and third mechanisms indicated strategic responses of plants involving several physiological mechanisms to cope with changing its surrounding environment. When plant maintained its water status either by efficient water absorption from roots or reducing transpiration with stomata closure could be termed under drought avoidance mechanism. As for drought tolerant, plants could withstand its stomata opening by maintaining its turgor and continued its metabolisms even under low water potential.

At present scenario of climate changes with elevated CO<sub>2</sub> in the atmosphere, it is predicted that drought could occur more frequent and severe in the future (IPPC, 2007). Therefore, it has paid attention in many researches to improve the agricultural productivity against the unfavorable environmental stresses (Sharma and Lavanya, 2002). In order to attain superior cultivar for the target environment, the most widely employed strategy is direct selection of high yielding cultivar in different location over years. However, this approach could not achieve further progress for improving a drought tolerant cultivar. Moreover, variations could encounter due to soil heterogeneity, environmental factors and their interactions.

With experiences and application of advanced technologies, integrated approach has been practiced including physiological studies to attain better yielding drought tolerant cultivar (Hammer and Jordan, 2007) However, drought tolerance or resistance of a plant species involved several physiological mechanisms (Nilsen and Orcutt, 1996; Chaves *et al.*, 2005) so it is a complex trait. Therefore, understanding the physiological response responses to drought is essential for a holistic perception of plant resistance mechanisms to water-limited conditions.

## 6. Some important physiological indicators for drought resistance

Considerable efforts have been directed towards identifying traits associated with drought resistance in various crops including soybean. Underlying the way of plant responses to drought, each mechanism implies as selection target or trait for crop improvement.

For drought escape, selection is aimed at those developmental and maturation traits that better calibrate water sensitive periods of crop ontogeny with the seasonally recurrent meteorological patterns of the targeted production area.

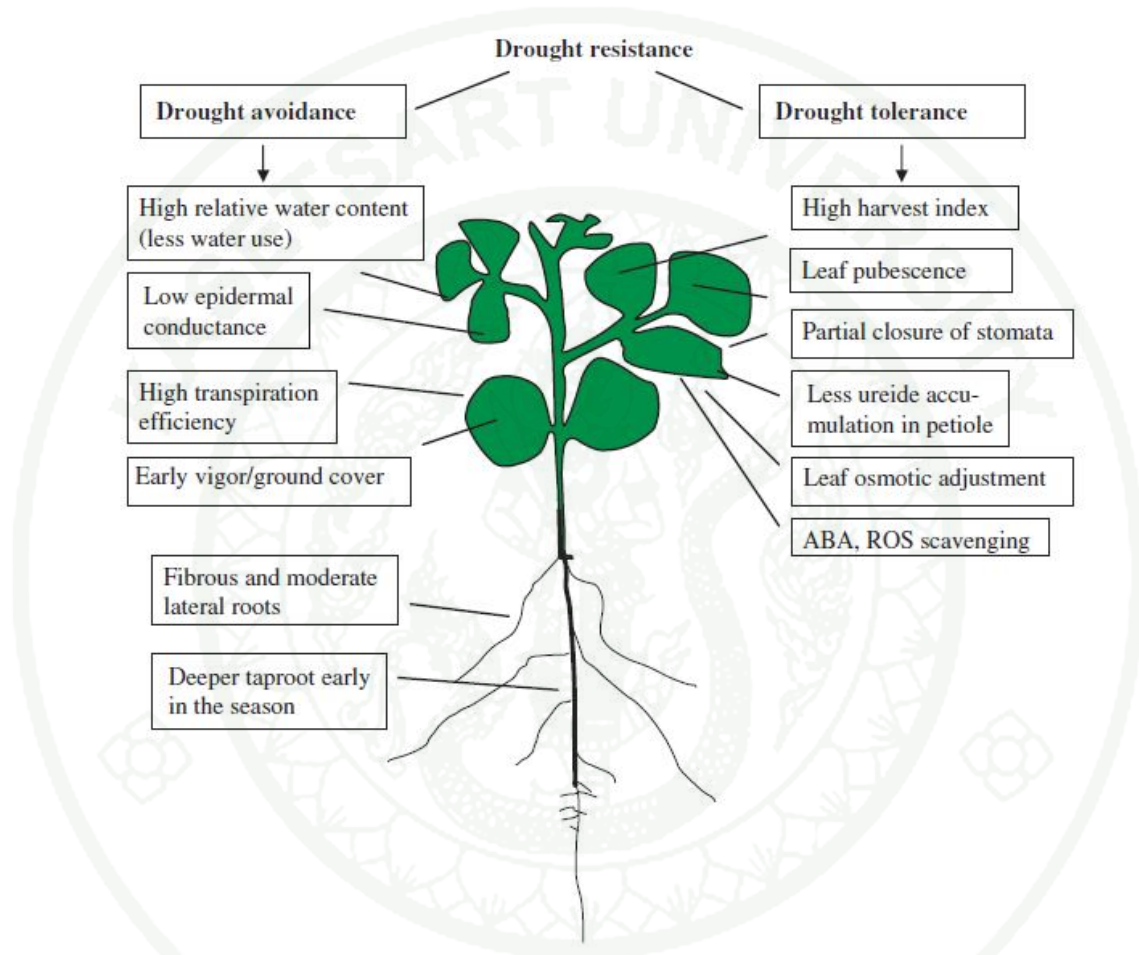
For dehydration avoidance or drought tolerance, selection is focused on traits that lessen evaporatory water loss from plant surfaces (Clawson *et al.*, 1986; Zhang *et al.*, 1992) or maintain water up take during drought via a deeper root system (Goldman *et al.*, 1989).

For dehydration tolerance or drought avoidance, selection is directed at maintaining cell turgor or enhancing cellular constituents that protect cytoplasmic proteins and membranes from desiccation.

Schilling and Misari (1992) reported that four techniques for evaluation of genotypes for drought resistance :are protoplasmic resistance to heat and dying, measurement of electrolyte escape, measurement of water loss from detached leaves, and rooting characteristics measured in rhizotron. Manavavalan *et al.* (2009) described the twelve major physiological traits related to the mechanisms of drought resistance in soybean (Figure 4).

Since, many physiological processes are interrelated, thus once cause may alter the others. Therefore, there is necessary to set some key parameters which could be conferred to other physiological indicators for crop improvement for drought resistance in soybean. In this study, electrolyte leakage of the leaf tissue (EL), specific

leaf weight (SLW), chlorophyll fluorescence, water use efficiency (WUE), root mass (RM) and biological nitrogen fixation (BNF) will be reviewed as major concerns.



**Figure 4** Traits associated with drought resistance in soybean.

**Source:** Manavalan *et al.* (2009)

### 6.1 Electrolyte leakage of the leaf tissue

Cell membranes are one of the first targets of many plants stress and it is generally accept that the maintenance of their integrity and stability under water stress conditions is a major component of drought tolerance in plants (Bajji *et al.*, 2001).

Measuring electrolyte leakage from leaf tissue has been long established technique to quantify damages to cell membranes in various abiotic stress conditions (Stoddard *et al.*, 2006) and biotic stress (Adam *et al.*, 2000; Sriram *et al.*, 2000) in several crop species. Electrolyte leakage is estimated by measuring the electrical conductivity of the medium which the leaf sample is equilibrated. The relative rate of this electrolyte leakage is used to estimate the cell membrane stability. Electrical conductivity (EC) of bathing solution of leaf tissue and EC % calculated from before and after killing the leaf tissue by freezing or autoclave (Chen *et al.*, 2006) are useful for determining to relative tissue leakage if cut area are constant and internal ion concentration in the leaf tissue are constant among samples (Whitlow *et al.*, 1992). The degree of cell membrane injury induced by water stress also could be also estimated through measurements of electrolyte leakage from the cells or plant tissue. It is suitable for drought tolerant rating in legumes species and cultivars (Grzesiak *et al.*, 1996; Deshmukh and Kushwaha, 2002).

It has been demonstrated recently that electrolyte leakage measurement may be correlated with several physiological and biological parameters conditioning the plant response to environmental conditions such as antioxidative enzyme synthesis (Liu and Hang 2000; Sreenivasulu *et al.*, 2000), membrane acyl lipid concentration (Lauriano *et al.*, 2000) water use efficiency (Franca *et al.*, 2000; Saelim and Zwiazek 2000), stomatal resistance, osmotic potential and leaf rolling index (Premachandra *et al.*, 1989). Therefore, the electrolyte leakage has been recommended as a valuable criterion for identification of stress resistant cultivars in several crop species (Bajji *et al.*, 2001).

## 6.2 Specific leaf weight

Since the 90% of organic matter is formed by the process photosynthesis. Therefore, traits related with leaf had been paid attentions as important indicators for selecting productive genotypes. Among the parameters of photosynthetic apparatus, leaf area duration (LAD) has been found to have the highest positive correlation with the grain yield (Bingham, 1971). Positive correlation between apparent

photosynthesis and specific leaf weight (SLW) in soybean had been reported (Well *et al.*, 1982). Genotypic difference in SLW (Nelson and Schweitzer, 1988) and apparent photosynthesis (AP) (Hesketh *et al.*, 1981; Weibhold *et al.*, 1981) have been demonstrated in soybean. Correlation between leaf thickness and WUE has been found in peanut genotypes (Wright and Rao, 1992). In additions, genotypes with high SLW had greater plant weight and greater water-use efficiency (WUE) (Brown and Byrd, 1997; Wright and Rao, 1992). Therefore, selection for high SLW may increase seed yield in soybean (Thompson *et al.*, 1995; 1996) and could be selected for the drought resistant genotypes (Wright and Rao, 1992). However, Ma *et al.* (1995) reported that correlation between SLW and photosynthesis rate was not significant and inconsistent across growth stages. Meanwhile, the significant correlation in specific leaf area (SLA) with leaf nitrogen content and Rubisco in peanut have been reported. SLA had negative correlation with SLW (Nageswara Rao *et al.*, 1995). In addition, higher photosynthesis on a leaf area (SLA) basis for leaves with high SLW is likely due to greater concentration of the photosynthetic apparatus such as chlorophyll, nitrogen, protein and enzymes (Hesketh *et al.*, 1981) per unit leaf area, and minerals concentration as well (Brown and Byrd, 1997). Degree of correlation between SLW, chlorophyll content, AP and seed yield differed with growth stages in soybean (Kokuban *et al.*, 1988).

### 6.3 Photosynthetic pigment composition and chlorophyll fluorescence

#### 6.3.1 Photosynthetic pigments composition

Photosynthetic pigments are colored complex organic molecules which absorb radiant energy in the visible range of visible spectrum (400-700 nm) and convert into chemical form of energy. There are eight types of chlorophylls but chlorophyll a and b are the most abundant in higher plants. In addition, carotenoids (carotene and xanthophyll) also associated with light absorption (400-500 nm) and energy transfer process therefore called as accessory photosynthetic pigments. Moreover, carotenoids prevent to chlorophyll from photo-oxidation damage as anti-oxidant defense system. Photosynthetic pigments, (chlorophylls and carotenoids) are

distributed in thylakoid membranes in the chloroplast of eukaryotes (Shinha, 2004; Taiz and Zeiger, 2006).

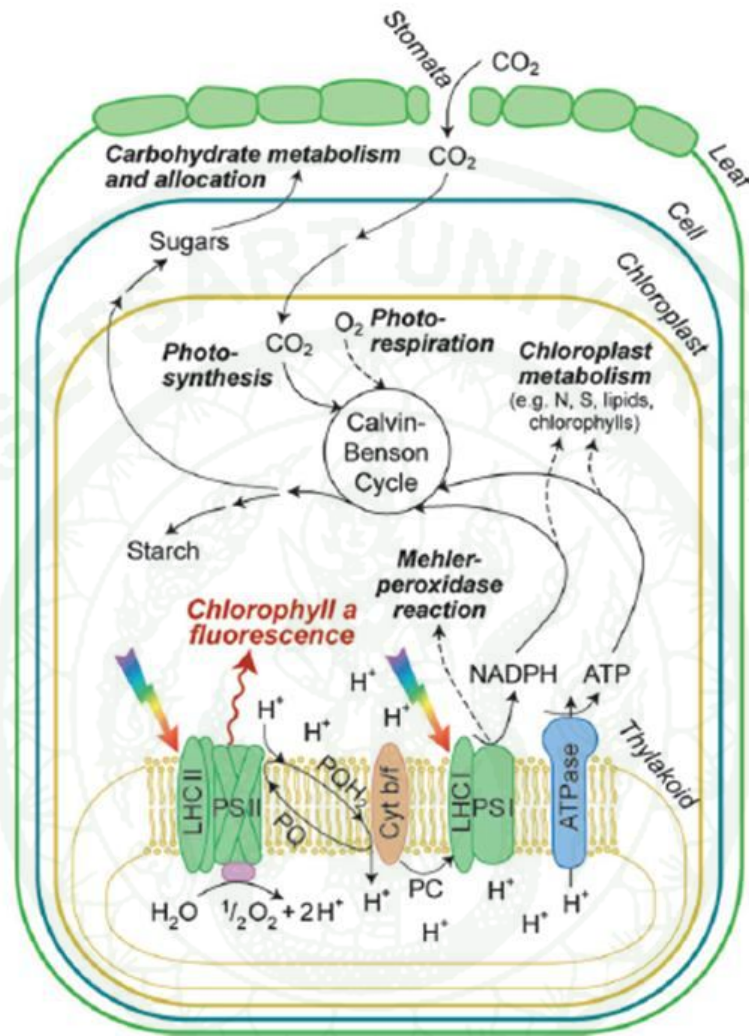
Generally, plants adjust leaf anatomy and physiology to its environment such as irradiance by developing thicker leaves with a greater mesophyll to surface-area ratio (Lichtenthaler *et al.*, 1981). In common adjustments, high irradiance also caused a reduction in the total Chl per unit leaf area and decreased Chl *a/b* ratio (Lichtenthaler *et al.*, 1981, Anderson, 1986). The Chl *a/b* ratio can be used as a bioassay to assess the light environment of a plant (Dale and Causton 1992) and drought stress as well (Reddy *et al.*, 2004; Jaleel *et al.*, 2009; Paknejad *et al.*, 2009). Genotypic variation in total chlorophyll content (Chl) and Chl *a/b* per leaf area was found in many crop species including soybean (Fritsch and Ray, 2007). Chlorophylls are primary components of photosynthesis process, therefore, quantitative analysis of chlorophylls and its activities have been paid attention to assess productive genotypes under different environments (Lu *et al.*, 2009; Paknejad *et al.*, 2009). Significant correlations between Chl and other physiological traits such as SLA, leaf N content (Nageswara Rao *et al.*, 2001), apparent photosynthesis rate (Butterry *et al.*, 1981) have been reported for many crop species including soybean (Thompson *et al.*, 1996; Ma *et al.*, 1995).

Commonly, photosynthetic pigments content could be estimated with spectrophotometry and fluorometry. In spectrophotometry, Chl from area known leaf pieces were extracted with various chemical solutions such as acetone (Arnon, 1949), NN-dimethylformamide (Moran, 1982) and dimethylsulphoxide (Hiscox and Israelstam, 1979), and estimated by subjected to spectrophotometer at specified wave length of light which absorbed by particular pigment. Recently, chlorophyll meter; SPAD (Soil Plant Analysis Development) has been widely used in estimation of Chl content. Numerous studies reported that SPAD reading had high correlation with Chl content and developed regression equations to estimate Chl content by measuring SPAD for several crops (Netto, 2005) including soybean (Fritsch and Ray, 2007).

### 6.3.2 Chlorophyll fluorescence in photosynthesis

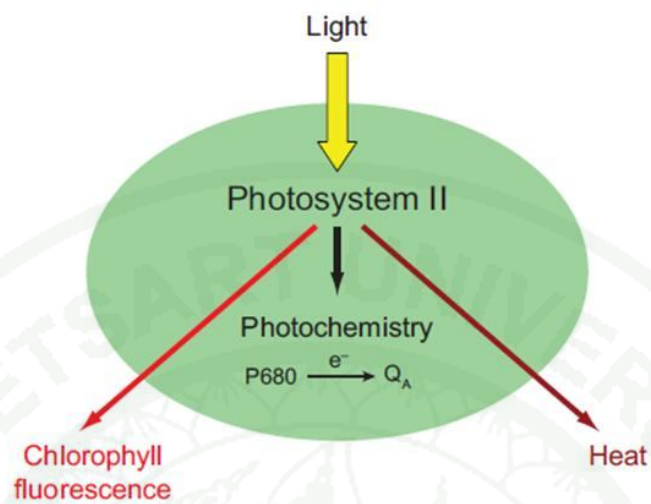
Photosynthesis is the only process in biology that harvesting of light and transform to chemical energy required for various metabolic processes. The most active photosynthetic tissue in the higher plants is the mesophyll of leaves. Mesophyll cells have many chloroplasts which contain the specialized light absorbing pigments. In the chloroplasts, absorbed light energy is converted into chemical energy by two different units called photo system I and II (PSI and PSII). Light harvesting and chemical transfer take place in PSII. Light induced oxidation of water (photolysis of water and oxygen evolution) takes part in energy transfer for PSII (Figure 5). Chemical energy converted form light energy is transported into PSI through various specialized proteins. PSI causes light induced reduction of  $\text{NADP}^+$  to NADPH and  $\text{H}^+$ . It is involved in both cyclic and non-cyclic photophosphorylation (The process of synthesis of ATP). The energy produced from PSI (NADPH and ATP) were transported to utilize in Calvin-Benson cycle and chloroplast metabolism (Shinha, 2004; Taiz and Zeiger, 2006). An overview of these processes was presented in the Figure 5.

When a photon of light collides with the capturing molecules (chlorophyll), an electron of outer most orbit of chlorophyll has increased vibrational energy which pushes an electron into high energy orbit causing activation of chlorophyll or excitation of chlorophyll (excited state) and an electron is completely ejected from the molecule to another chlorophyll molecule and being back to the ground state for another light (photon) absorption. Excited pigment may lose excitation energy in the forms of radiation (fluorescence), heat and chemical reactions or inductive resonance for another molecule (Figure 6). This process is called fluorescence (Shinha 2004; Taiz and Zeiger, 2006).



**Figure 5** Schematic representation showing the interactions of the main processes in C<sub>3</sub> photosynthesis in higher plants.

**Source:** Baker and Rosenqvist (2004)



**Figure 6** Simple model of the possible fates of light energy absorbed by photosystem II (PSII).

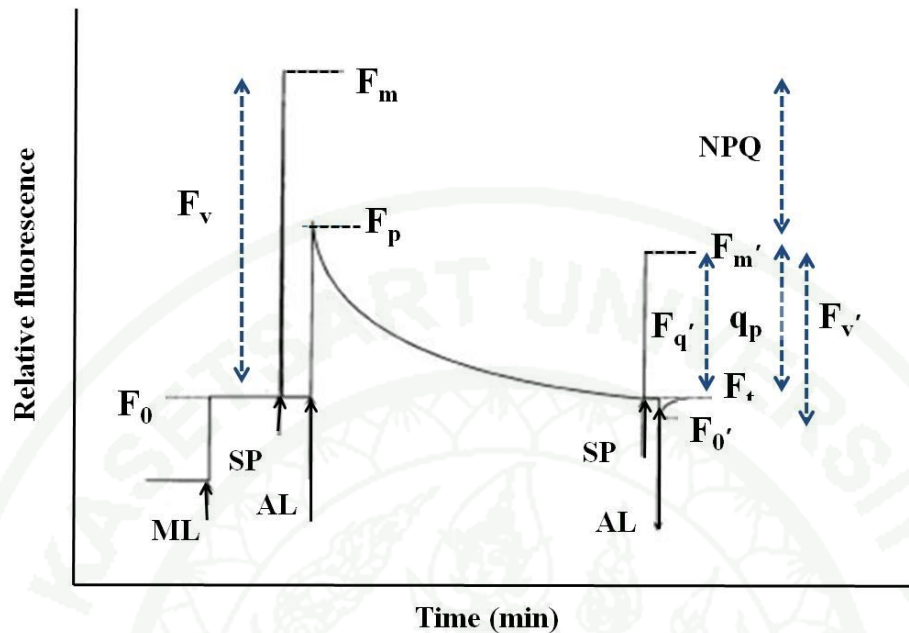
P680 is light harvesting chlorophyll proteins (LHCP) which absorbs up to 680 nm wave length of light

$Q_A$  is quinone which is primary electron acceptor of PSII.

**Source:** Barker (2008)

### 6.3.3 Chlorophyll fluorescence analysis

Photosynthesis performance of a crop species or genotype could be monitored and evaluated by measuring chlorophyll fluorescence from a dark adapted or light adapted leaves under a given environmental condition. Regarding to Maxwell and Johnson (2000), Rosenqvist and van Kooten (2003) and Baker (2008) modulated fluorescence is presented in the figure 7 as a brief.



**Figure 7** Kinetic curve of fluorescence induction sequence of typical fluorescence in dark adapted state and light adapted state of leaf and the basic chlorophyll fluorescence parameters. (Adapted from Maxwell and Johnson, 2000; Rosenqvist and van Kooten, 2003; Baker, 2008)

Where: ML; measuring light, SP; saturated pulse light, AL; actinic light,  $F_0$ ; minimal fluorescence (dark adapted),  $F_m$ ; maximum fluorescence (dark adapted),  $F_v$ ; variable fluorescence ( $F_m - F_0$ ),  $F_t$ ; Fluorescence at steady stated (light adapted),  $F_m'$ ; maximum fluorescence (light adapted),  $F_0'$ ; minimum fluorescence (light adapted),  $F_v'$ ; variable fluorescence ( $F_m' - F_0'$ );  $F_q'$  or  $q_p$ ; photosynthetic quenching of open PSII center ( $(F_m' - F_t) / (F_m' - F_0')$ ), NPQ; non photosynthetic quenching [ $(F_m / F_m') - 1$ ].

During the induction of photosynthesis when a dark-adapted leaf is exposed to weak light, large changes in chlorophyll fluorescence occur. On immediate exposure to a measuring light (ML), fluorescence rises to the minimal level ( $F_0$ ) of fluorescence (the fluorescence level when the PSII reaction centers are in the 'open' state and capable of photochemistry since  $Q_A$ , the primary quinone acceptor of PSII, is

maximally oxidized). Application of a saturating flash of light (SP) allows measurement of the maximum fluorescence level ( $F_m$ ). Turning on the actinic light (AL), typically in the presence of blue light (AL) drive photosynthesis (light adapted state) and fluorescence reach at  $F_p$ . When a leaf in the light-adapted state is exposed to a saturating flash light (SP), the maximum fluorescence in the light ( $F_m'$ ) obtained. The level of fluorescence immediately before the saturating flash light (SP) is termed  $F_t$ . After turning off the actinic light (AL), typically in the presence of far-red light, allows the zero level fluorescence 'in the light' to be estimated ( $F_0'$ ).

Based on above basic fluorescence, several fluorescence parameters have been risen related to interested in monitoring and evaluation on photosynthesis performance of a crop species or genotype with the field of concern under given environment (Baker and Rosenqvist, 2004; Clavel *et al.*, 2006). Maximum PSII efficiency of a given genotype or a crop species could be determined by  $F_v/F_m$  parameter under given environment including under drought condition (Baker and Rosenqvist, 2004). PSII operating efficiency (Baker, 2008) or  $\Phi_{PSII}$  (Maxwell and Johnson, 2000) can be estimated as  $[(F_q'/F_m')]$ ; where  $F_q'=(F_m'-F_t)$ . It estimates of the efficiency at which light absorbed by PSII is used for  $Q_A$  reduction photochemistry at given PPFd (light adapted). In addition, this parameter provides an estimate of the quantum yield of linear electron flux through PSII  $[(F_v'/F_m')]$ ; where  $[F_v' = (F_m' - F_0')]$  provides an estimate of the maximum efficiency of PSII photochemistry at given PPFd, which is the PSII operating efficiency if all the PSII center are opened ( $Q_A$  oxidized). Meanwhile, the parameter photochemical quenching  $[q_p; (F_m' - F_t) / (F_m' - F_0')]$ , estimates the proportion of electron transfer to PSII which could give additional indications that PSII are open to proceed its processes. Moreover, it reflects the redox state of PSII and the balance between energy supply and utilization (Ou *et al.*, 2003). In a contrary, NPQ  $[(F_m / F_m') - 1]$  estimates the non-photochemical quenching from  $F_m$  to  $F_m'$  and monitors the apparent rate constant for heat dissipation from PSII, and. Although the estimated quantum yield of PSII was proportionally related with electron transport rate which depending on  $CO_2$  assimilation, it could deviate due to chlorophyll content (Tsuyama *et al.*, 2003).

Since fixation of CO<sub>2</sub> process requires energy, therefore, assumed that electron transport rate (ETR) was proportionally related to CO<sub>2</sub> fixation under non-photorespiratory conditions. Electron transport rate (ETR) from PSII was estimated by  $\Phi_{PSII} \times PPFD \times 0.85$  (the mean light absorption of C<sub>3</sub> leaves)  $\times 0.5$  (approximate fraction of incident light absorbed light direct to PSII) (Rosenqvist and Kooten, 2003). However, a linear correlation was not always maintained under high light. Under water stress, the stomatal limitation imposed on photosynthesis under water deficits will be accompanied by a decreased in the rate of consumption of ATP and NADPH for CO<sub>2</sub> assimilation, which could result in decrease the rate of linear electron transport and, consequently PSII operating efficiency ( $F_q'/F_m'$ ) decreased. Flagella *et al.* (1998) showed the reduced quantum yield of PSII was associated with declined in Calvin cycle metabolism under drastic water deficits in cereal species (C<sub>3</sub> plants). In addition, water stress enhanced photo-inhibition and leading to oxidative damage therefore such magnitude of energy were utilized for anti-oxidation systems (Maxwell and Johson, 2000).

#### 6.4 Water use efficiency

Several evidences indicated that one mechanism for improving drought tolerance involves developing soybean lines or cultivars with higher WUE (Jones, 1993). Genetic variability for WUE has been found in cultivars or lines of several crop species including soybean (Mian *et al.*, 1996; 1998; Earl, 2002). WUE can be observed at several levels (Jones, 1992).

At a single leaf level, viz.  $WUE_{leaf}$  often defined as the ratio between instantaneous net CO<sub>2</sub> assimilation rate ( $A$ ) and transpiration ( $E_l$ ); instantaneous water vapor used for gas exchange. However, stomata are very asensitive responding to environmental changes that effected on gas exchange and transpiration, leading to variation in WUE. Therefore, it was suggested to approach with intrinsic water used efficiency ( $WUE_{in}$ ) while interested in improving WUE, for example through breeding (Jones, 2004).  $WUE_{in}$  defined as the ratio of net CO<sub>2</sub> assimilation rate ( $A$ )

and stomata conductance ( $g_s$ ), Evidences pointed out that  $WUE_{in}$  is rather constant over quite range of  $g_s$  (Chaves *et al.*, 2005). At whole plant level, viz.  $WUE_{plant}$  ( $WUE_t$ ) is determined as the ratio between rate of production and rate of plant transpiration ( $E_p$ ); is also known as transpiration efficiency. And, at a crop or vegetation scale, WUE can be expressed the ratio of production of total biomass (B), shoot biomass (S), or harvested yield (Y) against total evapotranspiration (ET) or plant transpiration ( $E_p$ ) (Jones, 2004).

At  $WUE_{leaf}$ , definition itself and numerous studies indicated clearly that WUE is primly determined by properties of stomata such as stomata conductance ( $g_s$ ), abilities of stomata closure. As brief theoretical concern; increasing osmotic pressure or decreasing water potential of guard cells by transport of potassium ion ( $K^+$ ) into guard cells regulate light-induced stomata opening and abscisic acid (ABA) transported from root regulates stomata closing are widely accepted. Mohammad and Naseem (2006) reported that stomata conductance increased with levels of  $K^+$  application.

$CO_2$  concentration in substomatal cavity or guard cells,  $SO_2$  concentration and pH of cytosol, mineral status, present of cytokinins in the plant system and environmental factors like temperature and light are affecting stomata movement (Shinha, 2004). Bunce (2000) demonstrated that stomata conductance of winter wheat and barley were significant affected by light, temperature, relative humidity and elevated  $CO_2$  levels. Numerous studies indicated that salinity of rhizospher (Yousif, *et al.*, 2010) and various exogenous chemicals application in various forms strongly affected on stomatal conductance (Tworkoski, *et al.*, 2002; Ibrahim, 2003). On the other hand, it has been reported that ABA content in xylem and leaf under drought stress was increased and then lead to decreasing stomatal conductance and turgor (Liu *et al.*, 2003). Stomatal sensitivity to a root-sourced ABA signal was gradually increased from the base to the apex, but .the stomata at the top of canopy can be more sensitively regulated in response to soil drying by enhancement of ABA with modification of pH of xylem along stem (Li *et al.*, 2010). Since various physiological

and biochemical mechanisms involved in stomata activities and interacting with environmental factors, therefore, it is very complex and dynamic.

Total leaf conductance is sum of  $g_s$  and epidermal conductance ( $g_c$ ). Therefore,  $g_c$  is also concerned for improving WUE, because when stomata tend to close the cuticular conductance may exceed the stomatal conductance in the water stress or dark adapted leaves, (van Gardngen and Grace, 1992; Boyer *et al.*, 1997). Under severe water deficit, stomata closure is maximized; thereby the total of cuticular conductance and any residual stomatal conductance determines the rate of water loss from leaf tissues. Species adapted to arid environments tend to have low leaf epidermal conductance ( $g_c$ ) (Helbsing *et al.*, 2000) and crop species or varieties with low  $g_c$  are often survive the longest under severe soil water deficit (Hull *et al.*, 1978; Sinclair and Ludlow, 1986; Jovanovi *et al.*, 1996). Intra-specific variation for minimum  $g_c$  has been identified in several species including soybean (Paje *et al.*, 1988). Generally, soybean cultivars adapted to drought which had greater WUE and lower leaf epidermal conductance ( $g_c$ ) (Hufstetler *et al.*, 2007).

Liu *et al.* (2003) reported that WUE at both single leaf and whole plant levels was improved at mild water deficit. At crop or vegetation scale, WUE can be improved by cultivar selection and innovation of irrigation and other cultural practices by understanding crop responses to water deficit at  $WUE_{leaf}$  and  $WUE_{plant}$  levels. Recently, it has been practiced regulated deficit irrigation and improved crop yields toward for water saving crop production (Zang *et al.*, 2006; Kang *et al.*, 2007; Demirtas *et al.*, 2010).

## 6.5 Root: development and distribution

Soybean root growth and development under field conditions consist of four stages (Hoogenboom *et al.*, 1987). Basically, (i) rapid root growth beneath plant rows during the vegetative stage, (ii) branching of roots during early reproductive growth, (iii) a decrease of root growth beneath rows and an increase of root growth

between rows during pod set, and (iv) cessation of root growth and root loss due to decomposition during physiological maturity.

It is commonly accepted that drought tolerant mechanisms in legume crops are closely related to the root system or rooting pattern (Pandey *et al.*, 1984; Itani *et al.*, 1992; Silim and Saxena, 1993). In soybean, significant correlations drought resistance and various root traits such as dry weight, total length, and volume and number of lateral roots (Liu *et al.*, 2005) had been reported.

The ability of a plant to change its root distribution in the soil is an important mechanism for drought avoidance. Sponchiado *et al.* (1980) showed that the ability of a plant to change root distribution to avoid drought stress could vary by cultivar within a species. Root growth and distribution patterns varied with soil water availability (Merrill *et al.*, 2002), soil physical and chemical properties (Ferrufino *et al.*, 2000) and cultural practices such as irrigation (Huck *et al.*, 1983), tillage and row spacing (Bohm *et al.*, 1977). In soybean, root growth of a cultivar or line varied with crop development stage (Manvalan *et al.*, 2010). Soybean has tap-root system, therefore, tap root elongation rate was also concerned for determination of deeper rooting ability which could lead larger root system during vegetative stage to withstand soil water deficit (Hirasawa *et al.*, 1994). Genotypic variation in tap root elongation rate was also found in soybean (Kaspar *et al.*, 1984). Matsui and Singh (2003) demonstrated that drought tolerant cowpea variety was associated with the increased in root dry matter per leaf area under mild water stress condition, and downward movement of roots under mild and severe water stress conditions at seedling stage. However, only a few studies have used them as screening criteria for drought tolerance because of the difficulties in visualizing root architecture.

#### 6.6 Biological nitrogen fixation tolerance in water deficit conditions

Among agronomic crops, the nitrogen requirement of soybean is high for vigorous plant growth and producing high-protein seeds (Sinclair and de Wit, 1975). Soybean plants attain required N from two sources; minerals N from soil and

atmospheric N through biological nitrogen fixation (BNF) process. BNF occurs in root-nodules of nodulated soybean via complex enzymatic systems. Primary nitrogen fixation enzyme, nitrogenase is present in bacteroids, floating in leghemoglobin, which takes oxygen (O<sub>2</sub>) regulation. The first stable product of N<sub>2</sub> fixation is ammonia, and then rapidly incorporated into amino acids via complex metabolic pathways catalyzed by many enzymatic systems before it is exported from nodules. Abundantly nodulated soybean plant is capable of fixing substantial amount of required N from BNF. The proportion of N derived from BNF varied from 0 to as high as 97 %, but most estimates fall between 25-75 %. (Keysher and Li, 1992). La Rue and Patterson (1981) reported that an average estimate of N<sub>2</sub> fixation in soybean to be 75 kg N ha<sup>-1</sup>, using average commercial yields. Bezdicsek *et al.* (1978) showed that soybeans are capable of fixing over 300 kg N ha<sup>-1</sup> when the soil is low in available N and effective strains of bradyrhizobia are supplied in high number.

N<sub>2</sub> fixation can be quantified with several techniques with various modifications. Basically, with the broad overview of the most commonly used methodologies to quantify N<sub>2</sub> fixating in soybean are; (1) Acetylene reduction method (King and Purcell, 2001; Streer, 2003; Sinclair *et al.*, 2007), (2) the N difference method (Israel and Mikkelsen, 2001; Saxena and Chandel, 1997), (3) <sup>15</sup>N dilution and abundance techniques (Boddey and Clark, 1984; Herridge *et al.*, 1990), (4) Ureides determination (McClure *et al.*, 1980; Sinclair *et al.*, 2000; Vandez and Sinclair, 2001).

The acetylene reduction assay (ARA) proved to be a sensitive diagnostic tool for detecting nitrogenase activity, and a useful technique for selecting species or cultivars for use as non-N<sub>2</sub> fixing reference plants. Potential N<sub>2</sub> fixation can be measured *in vivo* by exposing nodules or nodulated root to a very small partial pressure of acetylene in air, and then the amount of ethylene (C<sub>2</sub>H<sub>4</sub>) produced was measured by gas chromatography. ARA has been well established (Hardy *et al.*, 1968) and widely used to determined with various modifications for nitrogen fixation activity under concerned environment including water deficit conditions (Sinclair *et al.*, 2000; 2007; Stereer, 2003).

The N-difference procedure attempts to separate plant N into fractions originating from soil N or atmospheric N<sub>2</sub>. This is achieved by comparing the amount of N accumulated by the N<sub>2</sub> fixing plant under investigation with a neighboring non-N<sub>2</sub>-fixing control. The N present in the control is taken to represent a measure of the amount of soil mineral N available for plant growth during the growing season. It is assumed that the N<sub>2</sub> fixing species takes up the same amount of soil N. The amount of N<sub>2</sub> fixed is then calculated by difference: [N<sub>2</sub> fixed = (N yield fixing species) - (N yield non-fixing control)] The N-difference method is a simple approach that can be applied when facilities for only total N analyses are available. However, it can be varied due to differences in root morphology, residual concentration of inorganic N. Modifications to the N-difference method to include measurements of soil mineral N under the fixing and non-fixing control at the end of growth might improve estimates of BNF, but not if the inorganic N resulted from species differences in root turnover or rhizodeposition (Peoples *et al.*, 2002). In soybean, non-nodulation isolines were utilized in some N<sub>2</sub> fixation studies with this approach.

There are two stable isotopes of N; <sup>14</sup>N and <sup>15</sup>N. The heavy isotope of N (<sup>15</sup>N) occurs in atmospheric N. The technique was developed based on the differentiation between <sup>15</sup>N which derived from soil and derived from atmospheric N<sub>2</sub>. The difference in <sup>15</sup>N composition between soil N and N<sub>2</sub> is commonly increased for N<sub>2</sub> fixation studies by applying labeled <sup>15</sup>N-enriched (between 5 and 95 atoms% <sup>15</sup>N) inorganic or organic compounds to the soil. The various <sup>15</sup>N dilution and abundance techniques are commonly described, as 'isotope dilution techniques', since the unlabelled atmospheric N<sub>2</sub> that is biologically fixed 'dilutes' the <sup>15</sup>N labelled soil N taken up by the N<sub>2</sub>-fixing species during growth. It is the 'dilution' of <sup>15</sup>N in the fixing species relative to the non-fixing reference that provides the basis of the calculations used to provide a time-integrated (i.e.'average') estimate of %Ndfa (fraction of N difference) over a period of growth. This allows seasonal N<sub>2</sub> fixation to be calculated for an annual from a single analysis of the <sup>15</sup>N content of reference and N<sub>2</sub>-fixing species at around the time of peak biomass (Peoples *et al.*, 2002).

Allantoin and allantonic acid are the principal forms of nitrogen (N) transported in several legumes. The ureides, allatoin and allantoinate, are the final product of N<sub>2</sub> fixation that are exported from soybean's nodules to the shoot (McClure and Israel, 1979), where they are catabolized. The N contained in these ureides originates predominantly from N<sub>2</sub> fixation. Sinclair and Serraj, (1995) found that those species transporting nitrogen to the shoot in the form of ureides, such as soybean. Soybean lines, having N<sub>2</sub> fixation tolerance under drought condition were associated with low petiole ureides levels (Sinclair *et al.*, 2000). Nitrogenous compounds, ureides accumulation and metabolism in leaves (King and Purcell, 2005) have also been implicated in the feedback inhibition of N<sub>2</sub>-fixation under water limitation, therefore, has been applied as an indicator for selecting N<sub>2</sub> fixation drought tolerant soybean cultivars (Herridge, 1982; de Silva *et al.*, 1996; Serraj *et al.*, 1997; Purcell *et al.*, 1998; Sinclair *et al.*, 2003)

Nitrogen fixation is more sensitive to water deficits than many other processes (Serraj *et al.*, 1999), and this may restrict nitrogen supply and yield of soybean in many environments. An option to increase soybean yield is to eliminate or at least minimizes, the sensitivity of N<sub>2</sub> fixation to soil drying. An approach for making this improvement would be identified soybean genotypes that have greater N fixation tolerant to soil drying, and then to incorporate this trait to commercial cultivars.

Biological nitrogen fixation system was also severely depressed by increasing drought stress (Marino *et al.*, 2007). Under drought condition, nitrogen fixation activities in nodules was depressed (Ohashi, 2000), decreased in nodule size, number and nodule dry weight (Serraj *et al.*, 1999; Purcell *et al.*, 2000). Many efforts had done in investigation on response of N<sub>2</sub> fixation under drought conditions to resolute decreased of N<sub>2</sub> fixation under water stress conditions. Decreased in nitrogenase activity is primarily caused by nodule dehydration and tissue damage (Huang *et al.*, 1975; Albrecht *et al.*, 1984). Decreasing of photo-assimilates associated with reduced plant water status under drought and subsequently affected on N<sub>2</sub> fixation was well documented (Fellow *et al.*, 1984; Patterson and Hudak, 1996). Oxygen diffusion and sucrose substrate which mediated for N<sub>2</sub> fixation were limited under water deficits

(Cordon *et al.*, 1997; Gálvez *et al.*, 2005). In addition, both limitation of carbon flux and nitrogen accumulation in nodules involved reducing in N<sub>2</sub> fixation of soybean under drought stress (Streeter, 2003; Laudera *et al.*, 2007).

Larger nodule size is being conferred as important factor contributing to greater N<sub>2</sub> fixation (Purcell *et al.*, 1997; Serraj *et al.*, 1999). However, this, larger nodule size is partially contributing to greater in N<sub>2</sub> fixation under water deficit condition. Genetic variations in sensitivity of nodules numbers and dry weight to soil drying, and the responses of N<sub>2</sub> fixation rates to drought is related in part to nodule formation and growth have been reported (Serraj and Sinclair, 1996; 1998). High energy demand of N<sub>2</sub> fixation may create a greater sink demand by larger nodule than smaller nodule during water deficit, increase in phloem water supply to nodule, maintains nodule permeability to oxygen (O<sub>2</sub>), provides sugar to support nodule activity, and supply water for the export of urides from nodules. The relative drought insensitivity of N<sub>2</sub> fixation in drought tolerant cultivar Jackson was associated with high individual nodule dry weight under drought conditions. Drought tolerance of Jackson soybean cultivar is partially due to the advantages of large nodules, but that drought tolerance in Jackson also results from an inherently greater supply of photosynthates to nodules (King and Purcell, 2001). Nodule size and number are inversely related, and are determined by both host and Rhizobium strain genetically. There is a large range of diversity among grain legume species in the sensitivity of N<sub>2</sub> fixation to soil drying and genotypic variations in the sensitivity of N<sub>2</sub> fixation to soil drying also exist among soybean cultivars. Accumulated evidences indicated that cultivars having in greater in nodule numbers and larger in individual nodule size lead to increase in total nodule dry weight showed superior in N<sub>2</sub> fixation under different water regimes (Serrej and Sinclair, 1997; Sinclair *et al.*, 2007).

## MATERIALS AND METHODS

### Experiment I

#### Differential Responses of Selected Soybean Cultivars to Drought Stress and their Drought Tolerant Attributions

##### 1. Plant materials

The seven most cultivated soybean cultivars in Thailand, namely Nakhon Sawan 1(NS-1), SJ-4, Sukhothai 1(ST-1), Sukhothai 2(ST-2), Sukhothai 3(ST-3), Chiang Mai 60(CM-60) and Chakkrabandu 1(CK-1) were tested (Table 2). They were derived from various parents, and varied in maturity ranged from 80-120 days.

**Table 2** Soybean cultivars used for evaluation in the first experiment.

Soybean cultivar	Parentage	Physiological maturity (days)
Nakhorn Sawan 1(NS 1)	Doteung x Santamaria	80-90
SJ.4	Acadian x Tainung	100-110
Sukhothai 1(ST 1)	Shih Shih x SRF 400	100-110
Sukhothai 2 (ST 2)	7016 x Sukhothai 1	100-110
Sukhothai 3 (ST 3)	Fort Lamy x CM 60	90-100
Chiang Mai 60 (CM 60)	Willams x SJ.4	90-100
Ckakkrabandhu 1	UFC 1 x Santa Rosa	110-120

**Source :** Field Crops Research Institute, Department of Agriculture, Thainlad (2005) and Win *et al.*, (2009)

## 2. Crop management

Garden soil, enriched in nutrient was filled in plastic pots (25 cm in diameter and 25 cm in depth) up to 3.5 kg. Seeds were inoculated with commercial peat-based rhizobium inoculum prior to sowing. Nine seeds at three seeding spots were sown in a pot. Thinning was done at 11 days after sowing (DAS); maintaining three plants per pot which equivalent to 270,000 plants ha<sup>-1</sup>. Re-randomization was carried out twice a week from emergence until imposing treatments. Pots were equally watered by using measuring beaker except during observed period for stressed plants. Approximately 0.8 to 2.4 lit of water supplied to each pot at the time of watering depended on crop growth stage throughout crop growing.

## 3. Water treatments and experimental design

Water treatments were well watered (Control) and drought stress for 15 days which started at V<sub>3</sub> growth stage (V<sub>3</sub>) and then re-watered (Stress). Pots were equally watered by using measuring beaker in three-day interval, except for stressed pots during drought-stress period. The seven soybean cultivars and two water treatments were arranged in a randomized complete block design with three replications in greenhouse.

## 4. Data collection

Data collection were done at 0, 5, 10 and 15 days after V<sub>3</sub> growth stage (DAV<sub>3</sub>) of each cultivar under both water regimes which at V<sub>3</sub> and approximately reached at reproductive growth stage R<sub>2</sub>, R<sub>3</sub> and R<sub>4</sub> (Fehr *et al.*, 1977), repetitively.

### 4.1 Soil water status

Eighty cc of soil from water stress pots were taken at each sampling time during the stressed period. After weighting their wet weight, samples were dried

in oven at 80° C for 72 hr. Soil moisture percentage was calculated based on wet weight.

#### 4.2 Shoot tissue moisture content

Shoots of three plants from each pot were cut and sealed with plastic bags and kept in ice box to avoid dehydration. Samples were collected during 13:00-14:00 hr. In laboratory, surface cleaning was done with moisturized blotting tissue followed by immediate weighting on digital balance for their fresh weight. Samples were oven dried at 70° C for 72 hr. Percentage of shoot moisture content (STM %) was calculated from fresh weight and dried weight.

#### 4.3 Electrolyte leakage from leaf tissue

The first uppermost fully expanded leaf of each the plant was collected, put in zip-plastic bags and kept in ice box during sampling process. By avoiding major veins, 20 leaf-discs were obtained from each sample leaves using 6 mm diameter single punch. Leaf disc samples were placed in vials and washed 2 times with fresh de-ionized water to clean cell lysates. The capped vials containing 60 ml of fresh de-ionized water were incubated for 5 hr on the mechanical shaker at 30 rpm in dark. First electrolyte conductance was measured with digital electro-conductivity meter (Model: InoLab Cond Level 1). After the first measurement, the vials were autoclaved for at 105° C for 15 mins. After autoclave, the vials were left for 2 hr to cool down to room temperature, and then taken the second measurement. Along the processes of EL measurement, air-conditioned room temperature was kept around 22° C ( $\pm 2$ ). The percentage of electrolyte leakage (EL %) was calculated as the EL ( $\mu\text{S cm}^{-1}$ ) ratio between before and after autoclave (Chen *et al.*, 2006), which were subtracted to the concentration of fresh de-ionized water, then multiplication by 100.

#### 4.4 Shoot, root and nodule biomass

After obtained shoot biomass, roots samples were obtained by breaking the soil clod and gently shaking to root and obtained the root mass. Nodules were detached from roots after washing with tap water on 1 mm screen. After surface cleaning with blotting papers, followed by immediate weight on digital balance for their fresh weight and dried in oven-dried for 72 hr at 70° C for their dry weights.

Relative growth rate of shoot, root and nodule dry matter accumulation rate between 0-15 days after V<sub>3</sub> under both water regimes were calculated as follow (Bajji *et al.*, 2001):

$$(ln DWt_2 - ln DWt_1) / \text{Days interval between two observed times}$$

Where;

$ln DWt_2$  = natural logarithm of dry weight at the second observed time

$ln DWt_1$  = natural logarithm of dry weight at the first observed time.

#### 4.5 Drought tolerant attributions

At physiological maturity, plants were harvested and obtained seed yields In order to evaluate on drought tolerance of the tested soybean cultivars, drought tolerant indices were calculated as follows:

- 1) Stress Susceptibility Index (SSI): (Fischer and Maure, 1978);

$$SSI = \frac{1 - (Y_s / Y_p)}{SI}$$

Where: Stress Intensity;  $SI = 1 - (\bar{Y}_s / \bar{Y}_p)$

- 2) Mean Productivity (MP): (Roselle and Hamblin, 1981);

$$MP = \frac{(Y_s + Y_p)}{2}$$

- 3) Geometric Mean Productivity (GMP): (Roselle and Hamblin, 1981);

$$GMP = \sqrt{(Y_s)(Y_p)}$$

- 4) Stress Tolerance (TOL): (Roselle and Hamblin, 1981),

$$TOL = (Y_p - Y_s)$$

- 5) Stress Tolerance Index (STI): (Fernandez, 1992),

$$STI = \frac{(Y_s)(Y_p)}{(\bar{Y}_p)^2}$$

Where:  $Y_p$  = yield of a given genotype in non stress environment

$Y_s$  = yield of a given genotype in drought stressed environment.

$\bar{Y}_p$  = mean yield of all genotypes in non-stressed environment.

$\bar{Y}_s$  = mean yield of all genotypes in stressed environment.

## 5. Statistical analysis

Dry matter of shoot, root and nodules, EL%, STM %, Soil Moisture %, collected at 0, 5, 10 and 15 DAV<sub>3</sub>, and seed yields of the cultivars under two water regimes were subjected to analysis of variance (ANOVA) and treatment means were compared with least significant difference (LSD). The relationship among observed parameters of plant response and relationship among drought indices were analyzed by the use of simple correlation.

## 6. Place and time

The experiment was conducted in the greenhouse, the Central Laboratory and Greenhouse Complex, Kasetsart University at Kamphaengsaen, Nakhon Pathom, (14°01'N, 99°58'E.), during October, 2008 -January-2009.

## Experiment II

### Physiological Responses of Selected Soybean Cultivars to Progressive Soil Drying

#### 1. Plant materials

The three soybean cultivars namely Nakhorn Sawan 1(NS-1), Sukhothai 2(ST-2), Sukhothai 3(ST-3) were selected to investigate their physiological responses under different water regimes.

#### 2. Crop management

Soil from the first experiment was recycled. Pots were filled with the soil up to 3.75 kg. Seeds were inoculated with commercial peat-based rhizobium inoculum prior to sowing. Four seeds were sown in a pot, and left one plant after thinning at 11 days after sowing (DAS). Approximately 0.565 g of 8:24:24 (N:P:K) fertilizer was applied into single plant per pot after thinning. Re-randomization has carried out twice a week from emergence until imposing treatments. Prevention measures for pest and disease had taken out. Pots were equally watered by using measuring beaker at the time of watering except for stressed pots during observed period.

#### 3. Water treatments and experimental design

The three cultivars were tested under two water regimes; well watered (control) and rewatered after 20 days progressive soil drying (stress) which started at V<sub>3</sub> growth stage. They were arranged in randomized complete block with three replications. Pots were saturated with watering and sealed with black plastic bags prior to observation period, to prevent evaporation. During observation period, 100 ml of water added into control pots after pot weight measurement, daily.

#### 4. Data collection

Data were collected at 0, 5, 10, 15 and 20 days after V<sub>3</sub> growth stage (DAV<sub>3</sub>) of each cultivar under both water regimes which at V<sub>3</sub> and approximately reached at reproductive growth stage R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub> and R<sub>5</sub> (Fehr *et al.*, 1977), repetitively.

Shoot dry matter (SDM), specific leaf area (SLA), specific leaf weight (SLW), SPAD, chlorophyll content (Ch) were investigated at 0, 5, 10, 15, 20 DAV<sub>3</sub>. Soil moisture content, relative water content of the leaf (RWC), leaf photosynthesis and chlorophyll fluorescence were measured at 5, 10, 15 and 20 DAV<sub>3</sub> of the each cultivar from both water regimes. Solute concentration in the third upper most fully expanded leaf of each cultivar under both water regimes was measured at 2,5,7,9,11,14,17 and 19 days after imposing water treatments. Daily plant transpiration across observed period was recorded by measuring pot weight.

##### 4.1 Soil and plant water status

Soils samples from water stress pots were taken in 80 cc volume at each sampling time during the dried down period, after getting plant samples. After weighting their wet weight, samples were dried in oven at 80° C for 72 hr. Soil moisture percentage was calculated based on wet weight.

Plant transpiration from each tested cultivar was calculated from daily loss of pot weight under both water regimes during late noon time (17:00-18:00 hrs). The first upper most fully expanded leaf of each cultivar under both water regimes were collected during 13:00-14:00 hr and kept in zipped plastic bags and ice box. After measuring fresh weight (FW), their turgor weights (TW) were measured after soaking the pieces of the sample leaves in distilled water and left over night in refrigerator at 10° C in dark conditions. Leaf dry weight (DW) was obtained after drying the samples at 70° C for 72 hr. Relative water content of the leaf (RWC) was calculated as  $(FW-DW) \times 100 / (TW-DW)$ .

#### 4.2 Solute concentration in the leaf tissue and osmotic potential

Solute concentration in the third upper most fully expanded leaf of each cultivar under both water regimes was measured at 2,5,7,9,11,14,17 and 19 days after imposing water treatments. The samples were collected during 07:300-09:00 hrs. A piece of terminal leaflet from the third most fully expanded leaf of the plants from both water regimes were obtained and frozen by immersing in liquid nitrogen for 30 seconds and measured solute concentration (osmolarity) with osmometer (VAPRO). The osmotic potential ( $\psi_s$ ) was estimated from osmolality (solute concentration) by the van't Hoff equation:

$$\psi_s = (-RTCs)$$

where;

R is gas constant (8.32 J mol<sup>-1</sup> K<sup>-1</sup>)

T is the absolute temperature (in degree Kalvin or K)

Cs is the solute concentration of the solution.

#### 4.3 Photosynthesis capacity and chlorophyll fluorescence

Photosynthesis capacity ( $P_{max}$ ) of center leaflet of the second upper most fully expanded leaf of each cultivar under both water regimes was measured during mid-morning (08:30-10:30 hr) with portable photosynthesis system (*LI 6400: LICOR, Lincon.USA*) integrated with fluorometer (*LI-6400-40*). The leaf chamber of the meter was set at about 30° C and 70 % relative humidity (RH). All measurements at 5, 10, 15 and 20 days after imposing treatments from both water regimes were carried out at photon flux density (PFD) of 1200  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , with a constant air-flow rate of 400  $\text{mol m}^{-2} \text{s}^{-1}$ . The cuvette CO<sub>2</sub> concentration was set about 400  $\mu\text{mol mol}^{-1}$ .

The operating efficiency of PSII ( $F_q'/F_m'$ ), the maximum efficiency in quantum yield of PSII centers under given irradiation ( $F_v'/F_m'$ ), and the photochemical quenching [ $q_p = (F_m' - F_s) / (F_m' - F_0')$ ] were calculated from the measured parameters (Genty *et al.*, 1989). Electron transfer rate (ETR) from PSII was estimated by  $\Phi_{PSII} \times$

PFD x 0.85 (the mean light absorption of C<sub>3</sub> leaves) x 0.5 (approximate fraction of incident light absorbed light direct to PSII) (Rosenqvist and Kooten, 2005).

#### 4.4 SPAD reading and chlorophyll content

After photosynthesis fluorometer measurement, the sample leaves were collected with zipped plastic bags and placed in ice-box during samples time. Leaf disc (10 cm in diameter) was obtained from the center leaflet of each sample leaf with cork borer and recorded average of 5 SPAD (M 502) readings.

The Leaf disc of each sample was placed in glass tube and chlorophyll was extracted with 5 ml of N-N- Dimethylformamide by allowing incubation for 40 hr under dark condition at 10° C. The extract solution was subjected to spectrophotometer (SHIMADZU: UV-1601) to determine absorbance of chlorophyll a (Chl<sub>a</sub>) at 647 nm and at 664 nm for chlorophyll b (Chl<sub>b</sub>). After Chl extraction, Chl<sub>a</sub>, Chl<sub>b</sub> and total chlorophyll content (Chl<sub>t</sub>) were estimated according to Moran, (1982) as follows;

$$\text{Chl}_a = 12.64 A_{664} - 2.99 A_{647}$$

$$\text{Chl}_b = -5.6 A_{664} + 23.26 A_{647}$$

$$\text{Chl}_t = 7.04 A_{664} + 20.27 A_{647}$$

The values then converted into surface area basic as

$$\text{PC} = \text{Ch} (\text{V} / \text{A})$$

Where; PC = Particular chlorophyll concentration ( $\mu\text{g ml}^{-1}$ )

V = Used volume of N-N- Dimethylformamide

A = Used leaf surface area ( $\text{cm}^2$ )

The ratio of Chl<sub>a</sub> and Chl<sub>b</sub> was observed.

#### 4.5 Shoot biomass

Shoot biomass of each cultivar under both water regimes was collected at 0,5,10,15 and 20 days after imposing treatments and dried at 70° C for 72 hrs to obtain shoot dry matter (SDM).

#### 4.6 Water use efficiency (WUE)

Intrinsic water used efficiency at single leaf level ( $WUE_{in}$ ) of each cultivar under both water regimes was calculated from the data obtained from photosynthesis measurement.  $WUE_{in}$  is the ratio between the rate of light saturated photosynthesis ( $A_{max}$ ) and the stomata conductance ( $g_s$ ). In addition, water use efficiency at plant level ( $WUE_{plant}$ ) of each cultivar under both water regimes were also calculated as the ratio between the cumulative daily transpired water from the plant (from daily pot weight during observed period) and accumulated SDM during observed period.

#### 4.7 Seed yield

Seed yield (SdY) and its components of each cultivar were recorded at physiological maturity.

### 5. Statistical analysis

Collected data were subjected to analysis of variance (ANOVA) and treatment means were compared with least significant difference (LSD). The relationship among observed parameters of plant response and relationship among drought indices were analyzed by the use of simple correlation.

## 6. Place and time

The experiment was conducted in the greenhouse, the Central Laboratory and Greenhouse Complex, Kasetsart University at Kamphaengsaen, Nakhon Pathom, (14°01'N, 99°58'E.), during February- May, 2009.

### **Experiment III**

#### **Nodule Growth and Nitrogen Fixation of Selected Soybean Cultivars under Different Soil Water Regimes**

##### 1. Plant materials

The three soybean cultivars same as in the second experiment (NS-1, ST-2 and ST-3) were selected to examine their nodule growth and nitrogen fixation capacity under different water regimes.

##### 2. Crop management

Soil from the first experiment was recycled and pots were filled with the soil, up to 3.75 kg. Pots were washed several times by flash flooding to leach out residual  $\text{NO}_3^-$ . Seeds were inoculated with commercial peat-based rhizobium inoculum prior to sowing and 15 seeds were sown at five seeding. Thinning was done at 13 DAS and left 3 plants per pot which was equivalent to 270,000 plants  $\text{ha}^{-1}$ . Re-randomization has carried out twice a week from emergence until imposing treatments. Prevention measures for pest and disease had taken out. Pots were equally watered by using measuring beaker at the time of watering except for stressed pots during observed period.

### 3. Water treatments and experimental design

The three cultivars were tested under two water regimes; well watered (control) and re-watered after 15 days progressive soil drying (stress) which started at V<sub>4</sub> growth stage. They were arranged in randomized complete block design with three replications.

### 4. Data collection

Data collections were done at 0, 7, 12, 15 days after V<sub>4</sub> growth stage (DAV<sub>4</sub>) of the each cultivar and 7 days after re-watering (22 DAV<sub>4</sub>), which at V<sub>4</sub>; approximately reached at reproductive growth stage R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub> and R<sub>5</sub> (Fehr *et al.*, 1977), respectively.

#### 4.1 Soil and plant water status

Soil samples from water stress pots were taken in 80 cc at each sampling time during the dried down period. After weighting their wet weight, samples were dried in oven at 80° C for 72 hr. Soil moisture percentage was calculated based on wet weight.

The second most fully expanded leaves collected from each plant were collected to determine relative water content of the leaf (RWC). The procedure was followed as in the second experiment.

#### 4.2 SPAD (M-502) reading

Average of five SPAD reading (M-502) around mid-rib had taken on the each center leaf-left of the second most fully expanded leaves. The SAPD readings were taken before leaf samples collection for RWC measurement.

### 4.3 Shoot biomass

Shoots were collected at 0,7,12, 15 and 22 (DAV<sub>4</sub>) and dried in oven at 70° C for 72 hr.

### 4.4 Acetylene reduction assay, nodulated root and nodule

After shoot collection, Nodulated root samples were obtained by breaking the soil clod and gently shaking to the root mass and added into 750 CC glass bottles. Seventy-five ml of acetylene gas was injected to get gas-air mixture (1: 9) and incubated in laboratory under sufficient fluorescence light and controlled room temperature (25° C ± 1) for 30 minutes. Gas samples (10 cc) were taken out with syringe and saved in vacuette (vacum tube). Tip of the tube was sealed with para-film. The tubes were kept in refrigerator and subjected to gas chromatometer (HEWETT 5890 series II) equipped with a flame ionization detector to measured ethylene concentration in gas sample.

After incubation with gas mixture, root samples were washed with tap water on 1 mm screen and surface cleaned with blotting papers.

Nodules were detached and surface cleaning was done. Their numbers were recorded followed by immediately weighing with digital balance. Nodules samples were dried in oven at 70° C for 72 hr. Nodulated root collection and incubation had simultaneously done in replication by replication during 13:00-17:30 hr to keep freshness of the samples. Although nitrogenase activity in nodules declines slowly following removal of shoot from nodulated roots , immediate assay of nodulated roots over a short period and replication of the plant samples allows for meaningful analysis of treatment effects (Streeter, 2003).

#### 4.5 Total nitrogen concentration in the shoot biomass

Dried shoot samples at 7, 12 and 15 DAV<sub>4</sub> were ground to pass a 2 mm sieve and sub-sample was ground to pass 1 mm sieve and Total N in shoot was determined by micro-kjeldahl method.

Accumulated Total N in shoot biomass ( $\mu\text{g}$  of  $\text{g}^{-1}$  shoot dry matter) was calculated by multiplying shoot dry weight by shoot N concentration. Nitrogen and shoot biomass accumulation rate ( $\text{g plant}^{-1} \text{ day}^{-1}$ ) were determined by dividing total changes in changes in N and biomass ( $\text{g plant}^{-1}$ ) between two biomass harvests (7 and 17 DAV<sub>4</sub>). Nitrogen concentration in the accumulated biomass was determined by dividing the N accumulation rate by the biomass accumulation rate (King and Purcell 2006) that assumed derived from  $\text{N}_2$  fixation because soil was stipulated to leach out of mineral nutrients by flash flooding several times

In order to evaluate  $\text{N}_2$  fixation tolerance based on shoot N analysis was followed to (Sinclair *et al.*, 2000). The amount of N accumulated relative to the amount of biomass accumulated during particular period. Therefore, each of difference in N accumulated in the shoot between two biomass samples was divided by the difference in biomass between two harvests of sampling (7 and 15 DAV<sub>4</sub>). Consequently, the calculated value was divided for each cultivar by the N concentration of particular cultivar of the first harvest (7 DAV<sub>4</sub>). This calculation rested in a dimensionless tolerant ratio, which in the most case had a value of less than one. This tolerance ratio was interpreted to reflect the ability of each cultivar to sustain  $\text{N}_2$  fixation rate during the water deficit treatment so as to maintain the initial N concentration of the plant. A high value in this ratio was taken to indicate a potential tolerance of  $\text{N}_2$  fixation to soil drying.

#### 4.6 Seed yield

Seed yield (SdY) and its components of the cultivar were recorded at physiological maturity.

## 5. Statistical analysis

Collected data were subjected to analysis of variance (ANOVA) and treatment means were compared with least significant difference (LSD). The relationship among observed parameters of plant response and relationship among drought indices were analyzed by the use of simple correlation.

## 6. Place and time

The experiment was conducted in the greenhouse, the Central Laboratory and Greenhouse Complex, Kasetsart University at Kamphaengsaen, Nakhon Pathom, (14°01'N, 99°58'E.), during March- July, 2009.

# Experiment IV

## Field grown soybean yield varied with timing of drought stress occurrence

### 1. Plant materials and experimental design

The two soybean cultivars; NS-1 and ST-3 were tested under three water regimes which were arranged in randomized complete block design with four replications. The main plot factor was three water regimes; (1) irrigation maintained around field capacity throughout growing (control), (2) withholding water for 14 days started at V<sub>4</sub> (before flowering) and (3) withholding water for 14 days started at R<sub>4</sub>. In the drought water treatment, the irrigation was applied around field capacity except during imposing drought stress period. The irrigation was managed with drip irrigation system with controlled valve. The amount of water applied to each unit was recorded by water meter (m<sup>3</sup>).

## 2. Crop management

Seeds were dry dressed with peat based commercial rhizobium prior to sowing. Planting was managed at 30 cm for row spacing and 12.5 cm for plant spacing. Four to five seeds were sown at seeding. After germination, rhizobium peat soil suspension was poured into each row. Two plants per hill were left after thinning at 12 DAS and 113 gm of 0: 16: 8 compound fertilizer (8: 24: 24) was applied to each plot which equivalent to 120 kg ha<sup>-1</sup>. Planting density was maintained about 400,000 plants ha<sup>-1</sup>. Weeding was done twice at thinning and at canopy closed. Pest and diseases were taken care. Tensiometers were set in two replications of each water regimes at 20 and 40 cm depth.

## 3. Data collection

### 3.1 Some physical and chemical properties of soil of the experiment site

Some physical and chemical properties of the soil of the field were investigated. Volumetric soil moisture content against soil water potential was examined before planting with tensiometers.

### 3.2 Soil and plant water status

During drought stress period, the second most fully expanded leaf from the four plants were collected at 7 and 14 days after withholding water at V<sub>4</sub> for water treatment 1 and 2, and 4, 7 and 14 days after withholding water at V<sub>4</sub> for water treatment 1 and 2 with zippered plastic bags and placed in the ice-box to avoid dehydration during 14:00-16:00 hr. In the laboratory, 30 leaf discs from terminal leaflets of sample leaves were obtained with cork borer (13 mm in diameter) followed by immediate weight on digital balance for their fresh weight. The sample discs were added into glass bottles and soaked with 80 cc of distilled water and left over night in the refrigerator. The leaf discs were surfaced dried with blotting paper and then

weighted for their saturated weight and oven dried at 70° C for 72 hr for their weight. RWC was calculated from fresh weight, saturated weight and dry weight.

Daily soil water potential were recorded around 9:30-10:30 hr from tensiometers which were set in control and withholding water stress treatments throughout particular water stress period

### 3.3 SPAD reading and Electrolyte leakage from leaf tissue

The second most fully expanded leaf from the four plants were collected at 7 and 14 days after withholding water at V<sub>4</sub> for water treatment 1 and 2, and 4,7 and 14 days after withholding water at R<sub>4</sub> for water treatment 1 and 2 with zipped plastic bags and placed in the ice-box to avoid dehydration during 18:00-9:00 hr. In the laboratory, 20 leaf discs from center leaflets of sample leaves were obtained with cork borer (13 mm in diameter) and washed cell lysates with fresh de-ionized water two times and left for 5 hr as bathing on mechanical shaker at 3 rpm. The bathing solution was measured to determine electrolyte leakage ( $\mu\text{S cm}^{-2}$ ) with digital electro-conductivity meter (Model: InoLab Cond Level 1). Along the processes of EL measurement, air-conditioned room temperature was kept around 22° C ( $\pm 2$ ).

Average of five SPAD reading was recorded around mid rib of terminal leaflet of the second most fully expanded leaf before EL measurement.

### 3.4 Shoot biomass

Four plants from two hills were collected at 7 and 14 days after withholding water at V<sub>4</sub> for water treatment 1 and 2, and 7 and 14 days after withholding water at R<sub>4</sub> for water treatment 1 and 3 and oven dried at 70° C for 72 hr.

### 3.5 Yield and yield components

Sixteen plants from eight hills were harvested for yield components and plants from inner two rows (4.8 m<sup>2</sup>) were harvested to determine plot yield at physiological maturity.

#### 5. Statistical analysis

All collected data were subjected to analysis of variance, (ANOVA) and treatment means were compared with least significant difference (LSD).

#### 6. Place and time

The experiment was conducted in the Field of Agronomy Department, Kaetsart University at Kamphaengsaen, Nakhon Pathom, (14°01'N, 99°58'E.) during February, 2010-May, 2010.

## RESULTS AND DISCUSSION

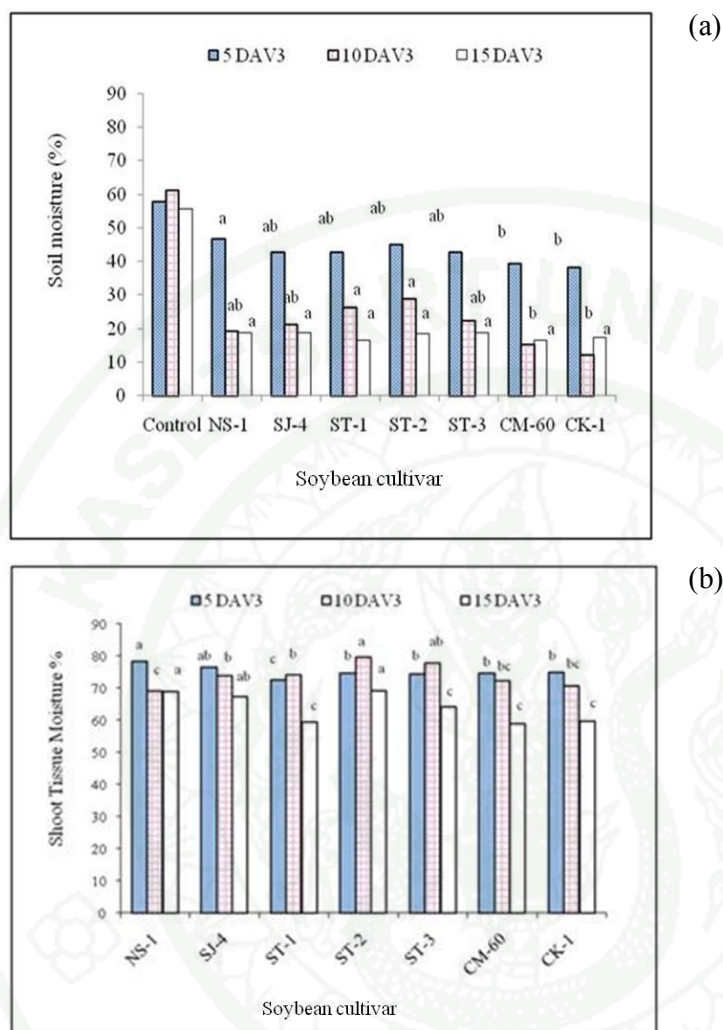
### Experiment I

#### Differential Responses of Selected Soybean Cultivars to Drought Stress and their Drought Tolerant Attributions

##### 1. Moisture status of soil and plants

Soil moisture in the pots of the control plants were maintained above 50% throughout observed period (0-15 DAV<sub>3</sub>.) meanwhile, soil moisture % (SM%) had fallen down under 47% under stress conditions since 5 DAV<sub>3</sub> and sharply declined at 10 DAV<sub>3</sub> (Fig 8a). However, SM% slowly declined at 15 DAV<sub>3</sub> due to lessen transpiration and additional watering (100 ml plant<sup>-1</sup>) to stress plants at 13 DAV<sub>3</sub> to avoid permanent wilting (Fig 8a). According to Wang *et al.* (2006), leaf water potential and transpiration of soybean plants dropped quickly when soil moisture below 47%, whereas net photosynthesis dramatically declined after 26%. Therefore, it was obvious that growth and development of plants were affected by imposed water stress in this study.

Ability to maintaining moisture in plant tissue is a key for its survival under water stress condition, therefore, shoot moisture of each tested soybean cultivar under both water regimes was determined (Fig 8b). Shoot tissue moisture content (STM%) of stressed plants were significantly lower ( $p < 0.0001$ ) than plants under control since 10 DAV<sub>3</sub>. Among tested cultivars, ST-2, NS-1 and SJ-4 maintained significantly higher STM% ( $p < 0.01$ ) at 69.02, 68.77, 67.30%, respectively, at 15 DAV<sub>3</sub> under prolonged water stress than other cultivars (Fig 8b).



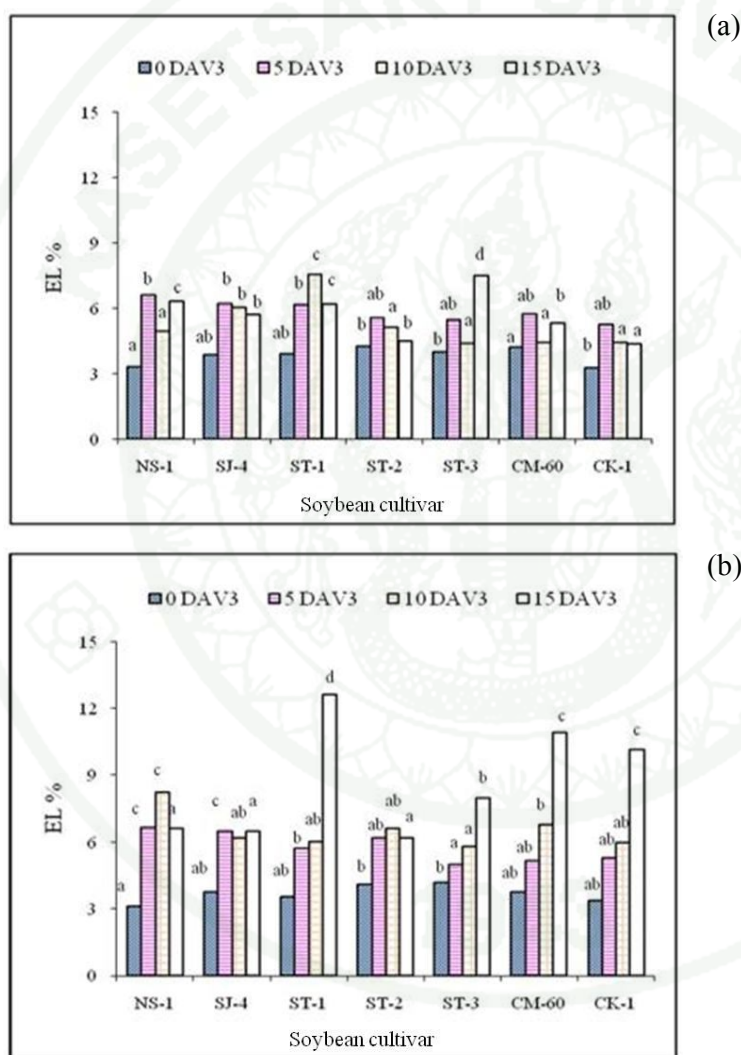
**Figure 8** Soil moisture depletion in drought stressed pots of different soybean cultivars compared with controlled pots (a). Shoot tissue moisture content of different soybean cultivar under drought-stressed regime (b) at 5, 10 and 15 DAV<sub>3</sub>.

Same letter above bar indicates not significant difference at LSD<sub>0.05</sub> at the observed time.

## 2. Electrolyte leakage

Cell membranes are one of the first targets of many plant-stress syndromes and it is generally accepted that the maintenance of their integrity and stability under

water stress conditions is a major component of drought tolerance in plants. Occurrence of stress stipulates cell membrane injuries and leads to increasing leakage of electrolytes. At 5 DAV<sub>3</sub>, percentage of electrolyte leakage (EL%) was not significant different between the two water regimes, but was turned significantly higher ( $p < 0.0001$ ) in the leaves of stressed plants since 10 DAV<sub>3</sub> (Fig 9).



**Figure 9** Electrolyte leakage of leaf tissue of different soybean cultivars under controlled water regime (a) and under drought-stressed water regime (b) at 5, 10 and 15 DAV<sub>3</sub>. Same letter above bar indicates not significant difference at LSD<sub>0.05</sub>.

The tested cultivars had already showed their genotypic differences at 0 DAV<sub>3</sub> and significant strong interaction between cultivars and water regimes resulted at 10 and 15 DAV<sub>3</sub>. Tsarouhas *et al* (2000) reported that values of EL% affected not only by genotypes, their age but also by level of stress intensity. In addition, Bajji *et al.* (2001) also reported that EL% could be varied due to minerals status of the plant which affected on solute concentration of cell sap. Nevertheless, the EL% values were consistent with STM%. ST-2, NS-1 and SJ-4 cultivars held higher STM% resulted in low EL% at the end of stress period. The results supported to the fact that better cell membrane integrity under stress conditions helped stabilizing moisture in plant parts and organelles that may result in maintaining stomatal conductance and osmotic potential (Premachandra *et al.*, 1989) which regulate transpiration, and consequently improve water use efficiency (Franca *et al.*, 2000), or vice visa.

## 2. Plant Growth and Development

Dry matter accumulation in shoot (SDM) significantly declined at 10 DAV<sub>3</sub> under drought stressed regime. At 15 DAV<sub>3</sub>, NS-1, SJ-4, ST-2 and ST-3 had greatest relative SDM at 78.59%, 77.13%, 78.76% and 72.45%, respectively (Table 3). In contrast, root dry matter accumulation (RDM) had increased in stressed plants at 10 DAV<sub>3</sub>; indicating that drought-stressed plants partitioned large amount of assimilates to root growth under water stress regime. At 15 DAV<sub>3</sub>, RDM significantly decreased, however NS-1, SJ-4 and ST-2 maintained root growth and not significant different from those under control water regime. Although the increase in RDM was maintained in ST-2, NS-1, and SJ-4, but it was decreased in other cultivars. At 15 DAV<sub>3</sub>, NS-1, SJ-4, ST-2 and ST-3 showed highest relative RDM compared with control water regimes at 107.02%, 96.77%, 116%, and 100% respectively (Table 4).

**Table 3** Shoot dry matter (g plant<sup>-1</sup>) of different soybean cultivars (Cr) at 0, 5, 10 and 15 DAV<sub>3</sub> under controlled (Cont) and drought-stressed (Str) water regimes (W).

Cultivar	0 DAV <sub>3</sub>		5 DAV <sub>3</sub>		10 DAV <sub>3</sub>		15 DAV <sub>3</sub>	
	Cont	Str	Cont	Str	Cont	Str	Cont	Str
NS 1	0.57 <sup>b</sup>	0.63 <sup>b</sup>	1.27 <sup>c</sup>	1.24 <sup>b</sup>	2.27 <sup>b</sup>	2.10 <sup>a</sup>	2.99 <sup>b</sup>	2.35 <sup>ab</sup>
SJ 4	0.55 <sup>b</sup>	0.60 <sup>b</sup>	1.18 <sup>c</sup>	1.20 <sup>bc</sup>	2.16 <sup>b</sup>	1.84 <sup>a</sup>	3.28 <sup>b</sup>	2.53 <sup>a</sup>
ST 1	0.68 <sup>b</sup>	0.63 <sup>b</sup>	1.22 <sup>c</sup>	1.00 <sup>bc</sup>	1.9 <sup>bc</sup>	1.79 <sup>ab</sup>	3.45 <sup>b</sup>	2.07 <sup>b</sup>
ST 2	0.62 <sup>b</sup>	0.5 <sup>b</sup>	0.98 <sup>c</sup>	1.09 <sup>bc</sup>	1.42 <sup>c</sup>	1.30 <sup>b</sup>	2.59 <sup>b</sup>	2.04 <sup>b</sup>
ST 3	0.52 <sup>b</sup>	0.45 <sup>b</sup>	0.93 <sup>c</sup>	0.89 <sup>c</sup>	1.49 <sup>c</sup>	1.37 <sup>b</sup>	2.65 <sup>b</sup>	1.92 <sup>b</sup>
CM 60	0.86 <sup>ab</sup>	0.88 <sup>a</sup>	1.59 <sup>b</sup>	1.50 <sup>a</sup>	2.45 <sup>a</sup>	2.19 <sup>a</sup>	3.96 <sup>a</sup>	2.26 <sup>ab</sup>
CK 1	1.05 <sup>a</sup>	1.02 <sup>a</sup>	2.05 <sup>a</sup>	1.80 <sup>a</sup>	2.95 <sup>a</sup>	2.15 <sup>a</sup>	4.47 <sup>a</sup>	2.46 <sup>a</sup>
Mean	0.69	0.69	1.32	1.25	2.09	1.65	3.34	2.23
CV %	16.9	14.5	17.4	12.5	14.0	17.1	15.2	7.8
LSD <sub>0.05</sub> (Cr)	0.21	0.18	0.42	0.31	0.52	0.41	0.90	0.31
LSD <sub>0.05</sub> (W)	0.07		0.14		0.16		0.24	
LSD <sub>0.05</sub> (Cr xW)	0.18		0.36		0.43		0.25	

In a column, means followed by the same letter are not significant different at LSD<sub>0.05</sub>

Greater NDM of ST-2, SJ-4 and NS-1 under drought-stressed (Table 5) resulted from their ability to maintain greater STM% through increasing root growth (Fig 8). Due to effect of imposed water stress, nodule growth was severely affected by drought stress and decreased up to 63 % of control (Table 5) while shoot growth was depressed up to 79.18% of control (Table 3) and root growth was increased up to 100.96% of control (Table 4).

**Table 4** Root dry matter ( $\text{g plant}^{-1}$ ) of different soybean cultivars (Cr) at 0, 5, 10 and 15 DAV<sub>3</sub> under controlled (Cont) and drought-stressed (Str) water regimes (W).

Cultivar	0 DAV <sub>3</sub>		5 DAV <sub>3</sub>		10 DAV <sub>3</sub>		15 DAV <sub>3</sub>	
	Cont	Str	Cont	Str	Cont	Str	Cont	Str
NS 1	0.15 <sup>a</sup>	0.15 <sup>a</sup>	0.35 <sup>a</sup>	0.32 <sup>b</sup>	0.44 <sup>b</sup>	0.55 <sup>a</sup>	0.57 <sup>ab</sup>	0.61 <sup>a</sup>
SJ 4	0.14 <sup>a</sup>	0.15 <sup>a</sup>	0.31 <sup>a</sup>	0.32 <sup>b</sup>	0.43 <sup>b</sup>	0.58 <sup>a</sup>	0.62 <sup>a</sup>	0.60 <sup>a</sup>
ST 1	0.11 <sup>b</sup>	0.09 <sup>c</sup>	0.29 <sup>a</sup>	0.27 <sup>c</sup>	0.40 <sup>b</sup>	0.43 <sup>b</sup>	0.61 <sup>a</sup>	0.55 <sup>a</sup>
ST 2	0.15 <sup>a</sup>	0.15 <sup>a</sup>	0.33 <sup>a</sup>	0.34 <sup>b</sup>	0.36 <sup>bc</sup>	0.44 <sup>b</sup>	0.50 <sup>a</sup>	0.58 <sup>b</sup>
ST 3	0.12 <sup>b</sup>	0.12 <sup>b</sup>	0.29 <sup>a</sup>	0.23 <sup>c</sup>	0.32 <sup>c</sup>	0.56 <sup>a</sup>	0.51 <sup>b</sup>	0.51 <sup>c</sup>
CM 60	0.10 <sup>c</sup>	0.10 <sup>bc</sup>	0.39 <sup>a</sup>	0.34 <sup>b</sup>	0.45 <sup>b</sup>	0.62 <sup>a</sup>	0.72 <sup>a</sup>	0.68 <sup>a</sup>
CK 1	0.12 <sup>b</sup>	0.11 <sup>bc</sup>	0.40 <sup>a</sup>	0.44 <sup>a</sup>	0.59 <sup>a</sup>	0.61 <sup>a</sup>	0.71 <sup>a</sup>	0.68 <sup>a</sup>
Mean	0.13	0.12	0.34	0.32	0.43	0.54	0.61	0.60
CV %	8.20	13.4	22.3	13.2	14.9	11.1	16.2	4.70
LSD <sub>0.05</sub> (Cr)	0.02	0.03	0.13	0.07	0.11	0.10	0.17	0.05
LSD <sub>0.05</sub> (W)	0.01		0.04		0.05		0.05	
LSD <sub>0.05</sub> (Cr xW)	0.02		0.11		0.12		0.12	

In a column, means followed by the same letter are not significant different at LSD<sub>0.05</sub>

NDM of all cultivar was not significantly different (Table 5) at 10 DAV<sub>3</sub>. However, NDM decreased dramatically at 15 DAV<sub>3</sub> under drought-stressed regime. At the end of stress period, NS-1, SJ-4 and ST-2 had highest relative NDM at 73.12%, 74.87% and 81.77% respectively.

**Table 5** Nodule dry (mg plant<sup>-1</sup>) of different soybean cultivars (Cr) at 0, 5, 10 and 15 DAV<sub>3</sub> under controlled (Cont) and drought-stressed (Str) water regimes (W).

Cultivar	0 DAV <sub>3</sub>		5 DAV <sub>3</sub>		10 DAV <sub>3</sub>		15 DAV <sub>3</sub>	
	Cont	Str	Cont	Str	Cont	Str	Cont	Str
NS 1	90.8 <sup>ab</sup>	88.8 <sup>b</sup>	123.0 <sup>b</sup>	116.4 <sup>b</sup>	166.2 <sup>ab</sup>	177.4 <sup>ab</sup>	234.4 <sup>ab</sup>	171.4 <sup>a</sup>
SJ 4	86.8 <sup>ab</sup>	87.9 <sup>b</sup>	133.4 <sup>ab</sup>	124.0 <sup>ab</sup>	177.2 <sup>ab</sup>	184.9 <sup>a</sup>	223.6 <sup>ab</sup>	167.4 <sup>a</sup>
ST 1	101.2 <sup>ab</sup>	86.7 <sup>b</sup>	138.4 <sup>ab</sup>	116.0 <sup>b</sup>	159.9 <sup>b</sup>	144.9 <sup>ab</sup>	252.6 <sup>ab</sup>	122.3 <sup>b</sup>
ST 2	67.3 <sup>b</sup>	66.3 <sup>b</sup>	99.2 <sup>c</sup>	100.9 <sup>b</sup>	134.6 <sup>b</sup>	126.6 <sup>b</sup>	187.1 <sup>b</sup>	153.0 <sup>ab</sup>
ST 3	85.1 <sup>b</sup>	85.0 <sup>b</sup>	110.9 <sup>b</sup>	112.0 <sup>b</sup>	165.3 <sup>ab</sup>	147.7 <sup>ab</sup>	277.7 <sup>a</sup>	153.8 <sup>ab</sup>
CM 60	110.8 <sup>a</sup>	120.2 <sup>a</sup>	134.4 <sup>a</sup>	141.4 <sup>a</sup>	188.3 <sup>ab</sup>	168.9 <sup>ab</sup>	270.0 <sup>a</sup>	160.1 <sup>ab</sup>
CK 1	109.8 <sup>a</sup>	119.2 <sup>a</sup>	147.2 <sup>a</sup>	150.2 <sup>a</sup>	203.2 <sup>a</sup>	169.4 <sup>ab</sup>	250.1 <sup>ab</sup>	134.1 <sup>b</sup>
Mean	93.0	93.4	126.6	123.0	170.7	160.0	242.2	151.7
CV %	14.5	9.9	14.6	14.0	13.2	19.8	18.4	11.6
LSD <sub>0.05</sub> (Cr)	24.0	16.5	22.2	30.73	40.2	56.28	79.3	31.31
LSD <sub>0.05</sub> (W)	7.84		11.02		17.23		22.09	
LSD <sub>0.05</sub> (Cr x W)	20.73		29.17		45.58		58.45	

In a column, means followed by same letter are not significant different at LSD<sub>0.05</sub>

The results of the dry matter accumulation rates of each cultivar during imposed observed period (0-15 DAV<sub>3</sub>) under both water regimes were shown in the table 6. The highest SDM accumulation rate found at NS-1 and SJ-4 and highest NDM accumulation rate found at NS-1, SJ-4, ST-2 and ST-3. In contrast, highest RDM accumulation rate resulted at ST-1, CM-60 and CK-1. While consideration on relative dry matter accumulation rates of shoot, root and nodule of each cultivar, NS-1, SJ-4, ST-2 and ST-3 showed highest SDM accumulation rate at 78.79%, 80.56%, 89.28% and 90.63% respectively. NS-1, SJ-4 and ST-2 showed highest relative RDM accumulation rate at 106.25%, 105.56%, 106.67% respectively and also obtained highest relative accumulation rate of NDM at 70.97%, 68.25% and 80.88% respectively.

**Table 6** Dry matter accumulation rate of shoot, root and nodule of different soybean cultivars during 0-15 DAV<sub>3</sub> under controlled (Cont) and drought-stressed (Str) water regimes.

Cultivar	Shoot (g plant <sup>-1</sup> day <sup>-1</sup> )		Root (g plant <sup>-1</sup> day <sup>-1</sup> )		Nodule (mg plant <sup>-1</sup> day <sup>-1</sup> )	
	Cont	Str	Cont	Str	Cont	Str
	NS-1	0.33 <sup>a</sup>	0.26 <sup>a</sup>	0.016 <sup>d</sup>	0.017 <sup>c</sup>	0.062 <sup>ab</sup>
SJ -4	0.36 <sup>a</sup>	0.29 <sup>a</sup>	0.018 <sup>c</sup>	0.019 <sup>b</sup>	0.063 <sup>ab</sup>	0.043 <sup>a</sup>
ST -1	0.32 <sup>a</sup>	0.24 <sup>ab</sup>	0.021 <sup>b</sup>	0.022 <sup>a</sup>	0.060 <sup>ab</sup>	0.022 <sup>b</sup>
ST -2	0.28 <sup>b</sup>	0.25 <sup>ab</sup>	0.015 <sup>d</sup>	0.016 <sup>c</sup>	0.068 <sup>ab</sup>	0.055 <sup>a</sup>
ST -3	0.32 <sup>a</sup>	0.29 <sup>a</sup>	0.017 <sup>cd</sup>	0.017 <sup>c</sup>	0.077 <sup>a</sup>	0.039 <sup>a</sup>
CM- 60	0.31 <sup>ab</sup>	0.19 <sup>b</sup>	0.024 <sup>a</sup>	0.022 <sup>a</sup>	0.060 <sup>ab</sup>	0.019 <sup>b</sup>
CK -1	0.29 <sup>b</sup>	0.18 <sup>b</sup>	0.023 <sup>a</sup>	0.022 <sup>a</sup>	0.055 <sup>b</sup>	0.008 <sup>b</sup>
Mean	0.32	0.24	0.019	0.019	0.064	0.033
CV %	10.3	15.5	9.6	10.7	19.0	29.7
LSD <sub>0.05</sub> (Cr)	0.06	0.07	0.0003	0.0004	0.022	0.017
LSD <sub>0.05</sub> (W)	0.002		0.001		0.008	
LSD <sub>0.05</sub> (Cr xW)	0.006		0.003		0.021	

In a column, means followed by the same letter are not significant different at LSD<sub>0.05</sub>

When consideration was taken into the relationship between SDM, RDM, NDM, their accumulation rates during 0 DAV<sub>3</sub> -15 DAV<sub>3</sub> (SAR<sub>0-15</sub>, RAR<sub>0-15</sub>, NAR<sub>0-15</sub>) with seed yield (SYd) (Table 7), SDM was significantly correlated with EL%, STM%, NDM and finally with SYd. Although RDM showed a significant correlation only with SDM, it may contribute indirectly to others since SDM had a significant and broad correlation with most of the observed parameters.

**Table 7** Correlation coefficient value of relationships between the observed parameters and seed yield (g plant<sup>-1</sup>) of the different soybean cultivars under different water regimes at 15 DAV<sub>3</sub>.

Parameter	SDM	RDM	NDM	SAR <sub>0-15</sub>	RAR <sub>0-15</sub>	NAR <sub>0-15</sub>	STM%	EL%
SYd	0.76**	0.21 <sup>ns</sup>	0.81**	0.60*	0.07 <sup>ns</sup>	0.49 <sup>ns</sup>	0.78**	-0.66**
SDM		0.57*	0.78**	0.45 <sup>ns</sup>	0.43 <sup>ns</sup>	0.35 <sup>ns</sup>	0.65*	-0.60*
RDM			0.15 <sup>ns</sup>	-0.36 <sup>ns</sup>	0.77**	-0.04 <sup>ns</sup>	-0.14 <sup>ns</sup>	-0.01 <sup>ns</sup>
NDM				0.67**	0.07 <sup>ns</sup>	0.48 <sup>ns</sup>	0.86**	-0.63*
SAR <sub>0-15</sub>					-0.37 <sup>ns</sup>	0.42 <sup>ns</sup>	0.82**	0.67**
RAR <sub>0-15</sub>						-0.25 <sup>ns</sup>	-0.33 <sup>ns</sup>	0.35 <sup>ns</sup>
NAR <sub>0-15</sub>							0.58*	-0.49*
STM%								-0.85**

SYd : seed yield (g plant<sup>-1</sup>), SDM: shoot dry matter, RDM; root dry matter, NDM; nodule dry matter; SAR<sub>0-15</sub>; shoot dry matter accumulation rate during 0-15 DAV<sub>3</sub>, RAR<sub>0-15</sub>; root dry matter accumulation rate during 0-15 DAV<sub>3</sub>, NAR<sub>0-15</sub>; nodule dry matter accumulation rate during 0-15 DAV<sub>3</sub>, STM%; shoot tissue moisture%, EL%; electrolyte leakage %of leaf tissue ns: not significant at  $p \leq 0.05$ ; \* significant at  $p \leq 0.05$  and \*\* : significant at  $p \leq 0.01$

NDM also significantly correlated with SDM, STM%, EL% and SYd as well. Moreover, there was a significant relationship between NDM and SAR<sub>0-15</sub> ( $r = 0.67^{**}$ ,  $p < 0.01$ ). In addition, maintaining STM% and membrane integrity resulted in significant correlations with SDM, NDM and SYd. Therefore, it was obvious that nodule growth and biological nitrogen fixation (BNF) depended not only on the ability in maintaining moisture status (Serraj and Sinclair, 1998; King and Purcell, 2001) but also the energy and carbon supplied by photosynthesis from the top parts (Fellows *et al.*, 1987). Moreover, the results agreed that nodule growth was more sensitive to drought stress than shoot growth regarding their relative growth under different water regimes.

### 3. Drought tolerance attributions of the cultivars

The yield based cultivar evaluation was also analyzed to support further breeding programs in this study. The stress tolerant attributes for the tested soybean cultivars estimated from Yp and Ys under the control and drought-stress regimes are presented in Table 8 and their relationship are shown in Table 9.

**Table 8** Mean of drought tolerance indices and seed yields (g plant<sup>-1</sup>) of tested soybean cultivars under controlled and drought-stressed (Stress intensity = 0.37) regimes.

Cultivar	Yp	Ys	MP	GMP	SSI	TOL	STI
NS-1	8.51c <sup>5</sup>	6.96b <sup>3</sup>	7.74bc <sup>6</sup>	7.67cd <sup>6</sup>	0.49a <sup>3</sup>	1.55a <sup>2</sup>	0.56bc <sup>6</sup>
SJ-4	10.93b <sup>4</sup>	9.10a <sup>1</sup>	10.02a <sup>1</sup>	9.89a <sup>1</sup>	0.35a <sup>2</sup>	1.82a <sup>3</sup>	0.92a <sup>1</sup>
ST-1	7.63c <sup>7</sup>	5.80b <sup>7</sup>	6.72c <sup>7</sup>	6.65d <sup>7</sup>	0.65a <sup>4</sup>	1.83a <sup>4</sup>	0.42c <sup>7</sup>
ST-2	8.47c <sup>6</sup>	7.86a <sup>2</sup>	8.16b <sup>5</sup>	8.14c <sup>5</sup>	0.19a <sup>1</sup>	0.61a <sup>1</sup>	0.63b <sup>4</sup>
ST-3	12.31ab <sup>2</sup>	5.63b <sup>7</sup>	8.97a <sup>3</sup>	8.31b <sup>4</sup>	1.47b <sup>7</sup>	6.68b <sup>7</sup>	0.65b <sup>3</sup>
CM-60	11.51ab <sup>3</sup>	5.82b <sup>5</sup>	8.66b <sup>4</sup>	8.15bc <sup>3</sup>	1.32b <sup>5</sup>	5.69b <sup>5</sup>	0.63b <sup>4</sup>
CK-1	12.88a <sup>1</sup>	6.38b <sup>4</sup>	9.63a <sup>2</sup>	9.04ab <sup>2</sup>	1.36b <sup>6</sup>	6.49b <sup>6</sup>	0.77ab <sup>2</sup>
LSD <sub>0.05</sub>	1.67	1.75	1.07	1.16	0.65	2.67	0.18

Yp: Seed yield under control water regime, Ys: seed yield under drought stress regime, MP: mean productivity, GMP: geometric mean productivity, SSI: stress susceptibility index, TOL: tolerance, STI: stress tolerance index. In a column, means followed by same letter are not significant different at LSD<sub>0.05</sub>. In a column, superscript-number shows rank of the cultivar.

All indices showed significantly correlation with seed yield under control water regime (Yp) and seed yield obtained under stressed water regime (Ys) (Table 8). However, cultivar rankings under particular index were different. SSI and TOL showed similar ranking of the cultivars. STI, GMP and MP showed similar ranking of cultivar, but different from former indices (Table 9).

Results of this study indicated that ST-1 was in the top rank due to TOL and SSI. However, ST-1 had low potential yield in both environments. Therefore, SSI and TOL failed to distinguish low yielding genotypes. Meanwhile STI, GMP and MP could distinguish better yielding genotypes. Moreover, SSI and TOL resulted in low potential in genetic aspects such as heritability, general combining ability and specific combining ability in wheat (Saba *et al.*, 2001).

**Table 9** Correlation coefficient among drought tolerance indices and seed yields under control and drought-stressed regimes.

	Yp	Ys	MP	GMP	SSI	TOL	STI
Yp	1	-0.20 <sup>ns</sup>	0.78 <sup>**</sup>	0.61 <sup>**</sup>	0.75 <sup>**</sup>	0.86 <sup>**</sup>	0.59 <sup>**</sup>
Ys		1	0.46 <sup>*</sup>	0.65 <sup>**</sup>	-0.79 <sup>**</sup>	-0.68 <sup>**</sup>	0.66 <sup>**</sup>
MP			1	0.97 <sup>**</sup>	0.17 <sup>ns</sup>	0.34 <sup>ns</sup>	0.96 <sup>**</sup>
GMP				1	0.06 <sup>ns</sup>	0.13 <sup>ns</sup>	0.99 <sup>**</sup>
SSI					1	0.98 <sup>**</sup>	0.09 <sup>ns</sup>
TOL						1	0.06 <sup>ns</sup>
STI							1

Yp: Seed yield under control water regime, Ys: seed yield under water stress regime, MP: mean productivity, GMP: geometric mean productivity, SSI: stress susceptibility index, TOL: tolerance, STI: stress tolerance index

ns: not significant at  $p \leq 0.05$ , \* ; significant at  $p \leq 0.05$ , \*\* : significant at  $p \leq 0.01$ .

Most studies with various crops pointed out that selection based on TOL and SSI identified the drought tolerant genotypes with low yield. But, MP, GMP and STI identified the drought tolerant genotypes with high yield (Fernandez, 1992; Sanjari and Yazdanehpas, 2008) and consistent correlation with yields in seasonal variations (Golabadi *et al.*, 2006) and locations (Pourdad, 2008). Therefore, STI was the most appropriate index for yield based selection followed by GMP and MP.

## Experiment II

### Physiological responses of Selected Soybean Cultivars under Watered and Progressive Soil Drying Water Regimes

#### 1. Soil and plant water status

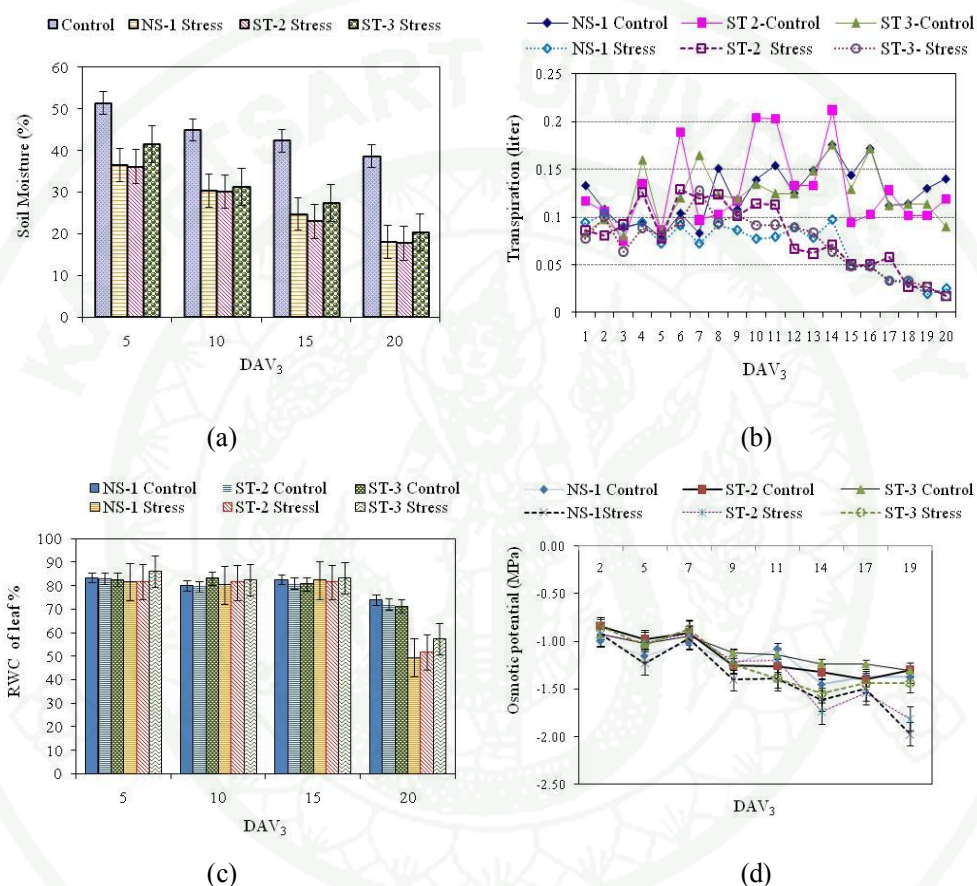
During sampling period, average of day and night temperature were 35.6° C ( $\pm 1.9$ ) and 23.7° C ( $\pm 1.3$ ). Relative humidity (RH) rose around 9-10:00 hr and reached 94.41% ( $\pm 2.3$ ) as maximum and thereafter sharply declined up to (40.65) ( $\pm 7.6$ ) as minimum around 16-17:00 hr of the day.

Soil moisture decreased with increasing dry period, and fall below 25% at the end of progressive soil drying (Fig 9a). The highest remaining soil moisture % (SM%) was observed in the soil of the pot of ST-3. Soil moisture in the soil of controlled plants also gradually decreased over the observed period due to cumulative insufficient water re-supply (100 ml day<sup>-1</sup>) to compensate plant transpiration which exceed over the amount of supplied water (Fig 9c).

Daily transpiration of control plants were fluctuated and ranged from 0.08-0.21 Lit day<sup>-1</sup>. Generally, daily transpiration of controlled plants, ranged from 0.9 to 2.1 ml day<sup>-1</sup> during observed period. Under progressive soil drying, transpiration rate of the cultivars were also fluctuated, but gradually declined after 11 DAV<sub>3</sub> and almost ceased at the end of dry down period (Fig 9c) while remaining SM% below 25%. All the tested soybean cultivars under soil drying maintained relative water content (RWC) of the leaf around 80% until 15 DAV<sub>3</sub> and not significant difference from controlled plants. However, significantly decreased at 20 DAV<sub>3</sub> (Fig 9c) compared to those of controlled plants. ST-3 maintained highest RWC at the end of dry down period (20 days) but not significant from other cultivars.

In soybean, solute concentration or osmotic potential ( $\psi_s$ ) played more important role for adaptation of water deficits in terms maintaining leaf turgor

pressure and regulation of water potential under field circumstances (Morgan, 1992). In this study, the highest  $\psi_s$  were found in leaves of ST-3 cultivar under both water regimes (Fig 10d).



**Figure 10** Soil moisture depletion in soil of the pot (a), daily plant transpiration (b) and relative water content of the leaf (c) osmotic potential development (d) of the soybean cultivars under watered (control) and progressive soil drying water regimes (stress) at observed time. Bars indicate s.e. (n=3)

## 2. Specific leaf area and specific leaf weight

Despite, specific leaf area (SLA) of the cultivars did not significantly reduce due to the effect of prolong soil drying (Table 10), but significantly reduced

their specific leaf weight (SLW) (Table 11). SLA of stressed plants slightly declined comparing to SLA of controlled plants (Table 10). Generally, both SLA and SLW of the cultivars decreased under progressive soil dry water regimes which were well documented. Under water stress, leaf enlargement (Boyer, 1970), leaf area and specific leaf area development (Shin *et al.*, 2005) were delayed and resulted in retarded growth. Sionit and Kramer (1977) reported that water stress decreased total leaf area and leaf weight. Pandey *et al.* (1984) also found that increasing drought stress progressively reduced leaf area, leaf area duration (LAD), crop growth rate (CGR), and shoot dry matter. Duration of epidermal cell division played in key role in this response at cell, organ and plant level (Gustavo *et al.*, 2008).

**Table 10** Specific leaf area (cm<sup>2</sup>) of the soybean cultivars (Cr) under watered (Control) and progressive soil dry (Stress) water regimes (W) at observed time.

Water regimes	Cultivar	Observed time				
		0 DAV <sub>3</sub>	5 DAV <sub>3</sub>	10 DAV <sub>3</sub>	15 DAV <sub>3</sub>	20 DAV <sub>3</sub>
Control	NS-1	41.2 <sup>a</sup>	81.0 <sup>a</sup>	71.1 <sup>a</sup>	72.3 <sup>ab</sup>	86.9 <sup>a</sup>
	ST-2	42.7 <sup>a</sup>	66.4 <sup>ab</sup>	68.7 <sup>a</sup>	86.5 <sup>a</sup>	77.6 <sup>a</sup>
	ST-3	38.0 <sup>a</sup>	49.8 <sup>b</sup>	46.9 <sup>b</sup>	57.4 <sup>b</sup>	69.3 <sup>a</sup>
Mean of control		44.6	65.8	62.2	72.1	77.9
Stress	NS-1	47.3 <sup>a</sup>	76.7 <sup>a</sup>	64.1 <sup>ab</sup>	66.1 <sup>a</sup>	85.9 <sup>a</sup>
	ST-2	43.8 <sup>a</sup>	65.8 <sup>ab</sup>	78.3 <sup>a</sup>	76.2 <sup>a</sup>	81.1 <sup>a</sup>
	ST-3	41.1 <sup>a</sup>	51.9 <sup>b</sup>	57.3 <sup>b</sup>	58.5 <sup>a</sup>	58.0 <sup>b</sup>
Mean of stress		44.5	64.8	66.6	66.9	75.0
LSD <sub>0.05</sub> (W)		6.39	14.05	9.24	10.89	11.63
LSD <sub>0.05</sub> (Cr)		7.83	17.21	11.31	13.34	14.25
LSD <sub>0.05</sub> (CrxW)		11.08	24.34	16.01	18.87	20.15
CV %		14.4	20.5	13.7	14.9	14.5

In a column, means followed by the same letter are not significant different at LSD<sub>0.05</sub>.

The cultivars showed genotypic difference in SLA and in SLW as well. SLW of the cultivars were not significant until 5 DAV<sub>3</sub> soil drying, by then showed significant decrease ( $p < 0.01$ ), comparing to SLW of control plants (Table 11). Among tested cultivars, ST-3 had smaller leaves in term of SLA, but greater in SLW than other two.

**Table 11** Specific leaf weight ( $\text{mg cm}^{-2}$ ) of the soybean cultivars (Cr) under watered (Control) and progressive soil dry (Stress) water regimes (W) at observed time.

Water regimes	Cultivar	Observed time				
		0 DAV <sub>3</sub>	5 DAV <sub>3</sub>	10 DAV <sub>3</sub>	15 DAV <sub>3</sub>	20 DAV <sub>3</sub>
Control	NS-1	2.81 <sup>b</sup>	3.04 <sup>b</sup>	3.73 <sup>b</sup>	4.45 <sup>a</sup>	4.31 <sup>a</sup>
	ST-2	2.92 <sup>b</sup>	2.88 <sup>c</sup>	3.52 <sup>b</sup>	3.56 <sup>b</sup>	4.01 <sup>a</sup>
	ST-3	3.90 <sup>a</sup>	3.23 <sup>a</sup>	5.12 <sup>a</sup>	4.11 <sup>a</sup>	4.07 <sup>a</sup>
Mean of control		3.21	3.05	4.12	4.04	4.36
Stress	NS-1	3.16 <sup>a</sup>	2.91 <sup>b</sup>	3.77 <sup>b</sup>	3.92 <sup>a</sup>	3.32 <sup>ab</sup>
	ST-2	3.01 <sup>a</sup>	2.95 <sup>b</sup>	3.21 <sup>c</sup>	3.12 <sup>b</sup>	2.88 <sup>b</sup>
	ST-3	3.90 <sup>a</sup>	3.31 <sup>a</sup>	4.05 <sup>a</sup>	4.10 <sup>a</sup>	3.85 <sup>a</sup>
Mean of stress		3.28	3.06	3.68	3.72	3.52
LSD <sub>0.05</sub> (W)		0.26	0.11	0.17	0.32	0.26
LSD <sub>0.05</sub> (Cr)		2.11	1.26	2.03	2.41	2.34
LSD <sub>0.05</sub> (CrxW)		0.45	0.19	0.29	0.55	0.45
CV %		7.6	3.4	4.1	7.8	6.5

In a column, means followed by the same letter are not significant different at LSD<sub>0.05</sub>.

### 3. SPAD reading and chlorophyll content

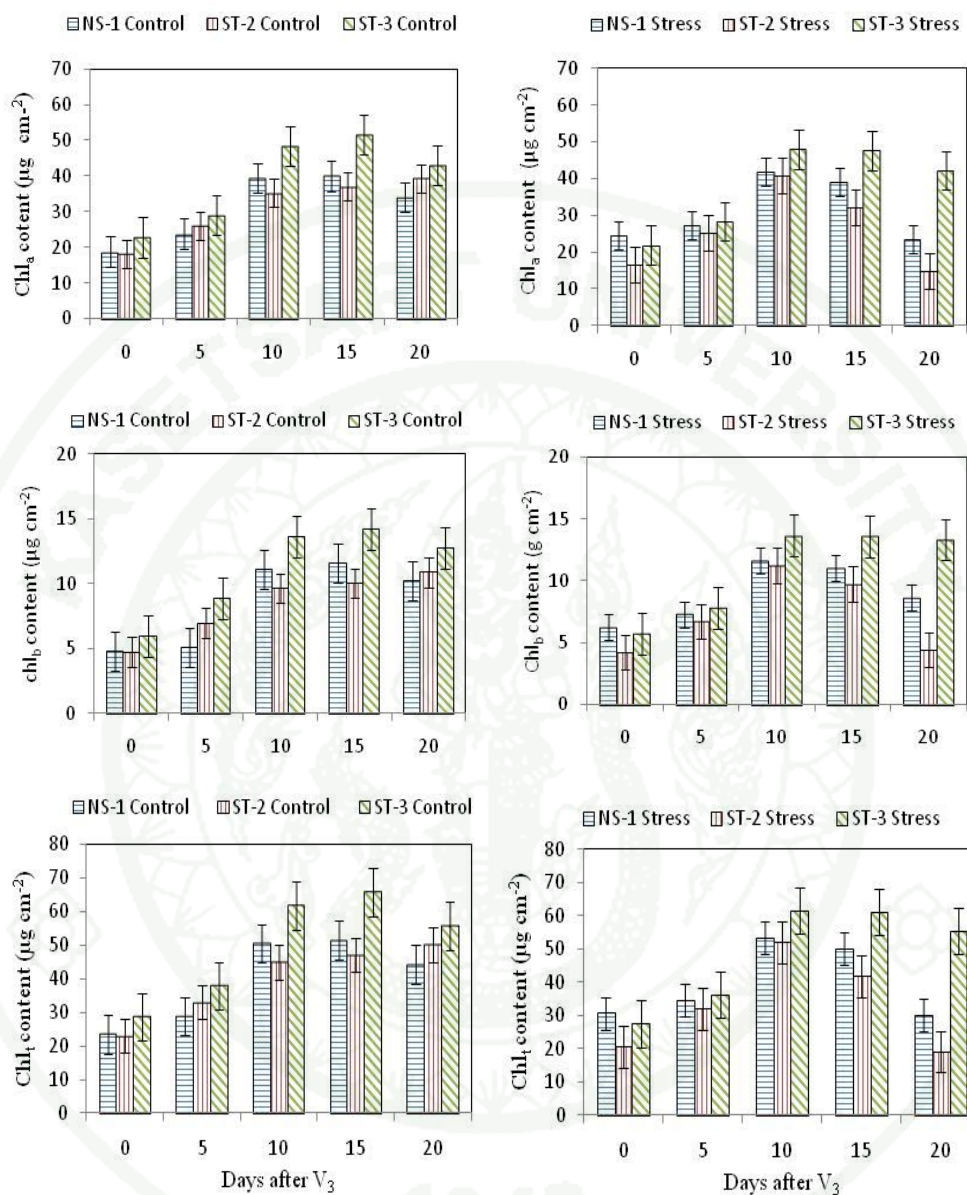
Chlorophyll meter has been widely use to estimate chlorophyll content in leaves of crop species (Cassol *et al.*, 2008) and nitrogen status as well because of the

majority of leaf nitrogen contributed to chloroplast (Hák *et al.* 1993). Significant correlations between SPAD reading and Chl have been reported in many crop species including soybean (Fritschi and Ray. 2007). In this study, SPAD reading increased at early period of soil dry, but significantly declined ( $p < 0.01$ ) at the end of prolong soil drying (Table 12). There was no genotypic difference among tested cultivars in SPAD reading under control water regime, generally. However, significant different results among the cultivars were found under prolong soil drying at 20 DAV<sub>3</sub> and ST-3 showed greater SPAD reading under soil drying water regime (Table 12).

**Table 12** SPAD reading of the leaf of the soybean cultivars (Cr) under watered (Control) and progressive soil dry (Stress) water regimes (W) at observed time.

Water regimes	Cultivar	Observed time				
		0 DAV <sub>3</sub>	5 DAV <sub>3</sub>	10 DAV <sub>3</sub>	15 DAV <sub>3</sub>	20 DAV <sub>3</sub>
Control	NS-1	32.5 <sup>a</sup>	34.7 <sup>b</sup>	40.6 <sup>a</sup>	41.7 <sup>ab</sup>	37.5 <sup>a</sup>
	ST-2	28.4 <sup>a</sup>	37.1 <sup>a</sup>	37.8 <sup>a</sup>	38.4 <sup>b</sup>	39.1 <sup>a</sup>
	ST-3	30.6 <sup>a</sup>	38.2 <sup>a</sup>	40.7 <sup>a</sup>	42.9 <sup>a</sup>	39.0 <sup>a</sup>
	Mean of control	30.5	36.7	39.7	41.0	38.6
Stress	NS-1	28.9 <sup>a</sup>	38.6 <sup>a</sup>	40.3 <sup>a</sup>	40.6 <sup>a</sup>	29.8 <sup>b</sup>
	ST-2	27.8 <sup>a</sup>	35.6 <sup>b</sup>	38.8 <sup>a</sup>	37.3 <sup>a</sup>	27.7 <sup>b</sup>
	ST-3	32.8 <sup>a</sup>	36.7 <sup>ab</sup>	40.2 <sup>a</sup>	41.0 <sup>a</sup>	35.6 <sup>a</sup>
	Mean of stress	29.8	37.0	39.7	39.6	30.64
LSD <sub>0.05</sub> (W)		2.89	1.26	2.03	2.41	2.31
LSD <sub>0.05</sub> (Cr)		3.54	1.54	2.49	2.95	2.37
LSD <sub>0.05</sub> (CrxW)		5.01	2.18	3.52	4.17	3.86
CV %		9.1	3.3	4.9	5.7	6.1

In a column, means followed by the same letter are not significant different at LSD<sub>0.05</sub>.



**Figure 11** Chlorophyll a, b and total chlorophyll content of leaf of the cultivar under watered (right) and progressive soil dry stress (left) water regimes. Bars indicate s.e (n=3).

Similar trends were found in chlorophyll contents (Fig 11) of the leaf as SPAD. However, contrasting to SPAD, the fact that Chl content was significantly declined since 15 DAV<sub>3</sub> due to the soil moisture depletion whereas SPAD did not.

Both Chl<sub>a</sub> and Chl<sub>b</sub> slightly increased under soil dry water regime over control at 10 DAV<sub>3</sub>, but declined under control later and significantly reduce at 20 DAV<sub>3</sub> (Fig 11). Chl<sub>a</sub> reduced 31.92% and Chl<sub>b</sub> reduced 21.92% of the leaf of the controlled plants, respectively at 20 DAV<sub>3</sub>. Therefore, Chl<sub>a</sub> was much more affected than Chl<sub>b</sub> by moisture depletion. As a result of Chl<sub>a</sub> and Chl<sub>b</sub> reduction, Chl a/b ratio significantly decreased since 15 DAV<sub>3</sub> (Table 13) under depleted soil moisture condition.

**Table 13** The ratio of chlorophyll a and b of the leaf of the soybean cultivars (Cr) under watered (Control) and progressive soil drying (Stress) water regimes (W) at observed time.

Water regimes	Cultivar	Observed time				
		0 DAV <sub>3</sub>	5 DAV <sub>3</sub>	10 DAV <sub>3</sub>	15 DAV <sub>3</sub>	20 DAV <sub>3</sub>
Control	NS-1	3.91 <sup>a</sup>	4.72 <sup>a</sup>	3.47 <sup>a</sup>	3.47 <sup>b</sup>	3.34 <sup>a</sup>
	ST-2	3.87 <sup>a</sup>	3.75 <sup>b</sup>	3.61 <sup>a</sup>	3.68 <sup>a</sup>	3.64 <sup>a</sup>
	ST-3	3.87 <sup>a</sup>	3.31 <sup>c</sup>	3.63 <sup>a</sup>	3.63 <sup>a</sup>	3.38 <sup>a</sup>
	Mean of control	3.88	3.93	3.58	3.59	3.45
Stress	NS-1	3.93 <sup>a</sup>	3.76 <sup>a</sup>	3.55 <sup>a</sup>	3.55 <sup>a</sup>	2.81 <sup>ab</sup>
	ST-2	3.92 <sup>a</sup>	3.75 <sup>a</sup>	3.30 <sup>b</sup>	3.30 <sup>b</sup>	3.29 <sup>a</sup>
	ST-3	3.83 <sup>a</sup>	3.64 <sup>a</sup>	3.52 <sup>a</sup>	3.52 <sup>a</sup>	3.16 <sup>a</sup>
	Mean of stress	3.89	3.72	3.58	3.45	3.09
LSD <sub>0.05</sub> (W)		0.160	0.650	0.107	0.106	0.235
LSD <sub>0.05</sub> (Cr)		0.196	0.797	0.132	0.130	0.311
LSD <sub>0.05</sub> (CrxW)		0.277	0.127	0.186	0.184	0.439
CV %		3.9	16.2	2.9	2.9	7.4

In a column, means followed by the same letter are not significant different at LSD<sub>0.05</sub>.

At 20 DAV<sub>3</sub>, both of Chl<sub>a</sub> and Chl<sub>b</sub> dramatically declined. As a result, Chl<sub>t</sub> significantly reduced under progressive soil dry water regime. Paknejad *et al.* (2009) also reported that Chl<sub>t</sub> content was affected by water stress at 70% soil moisture

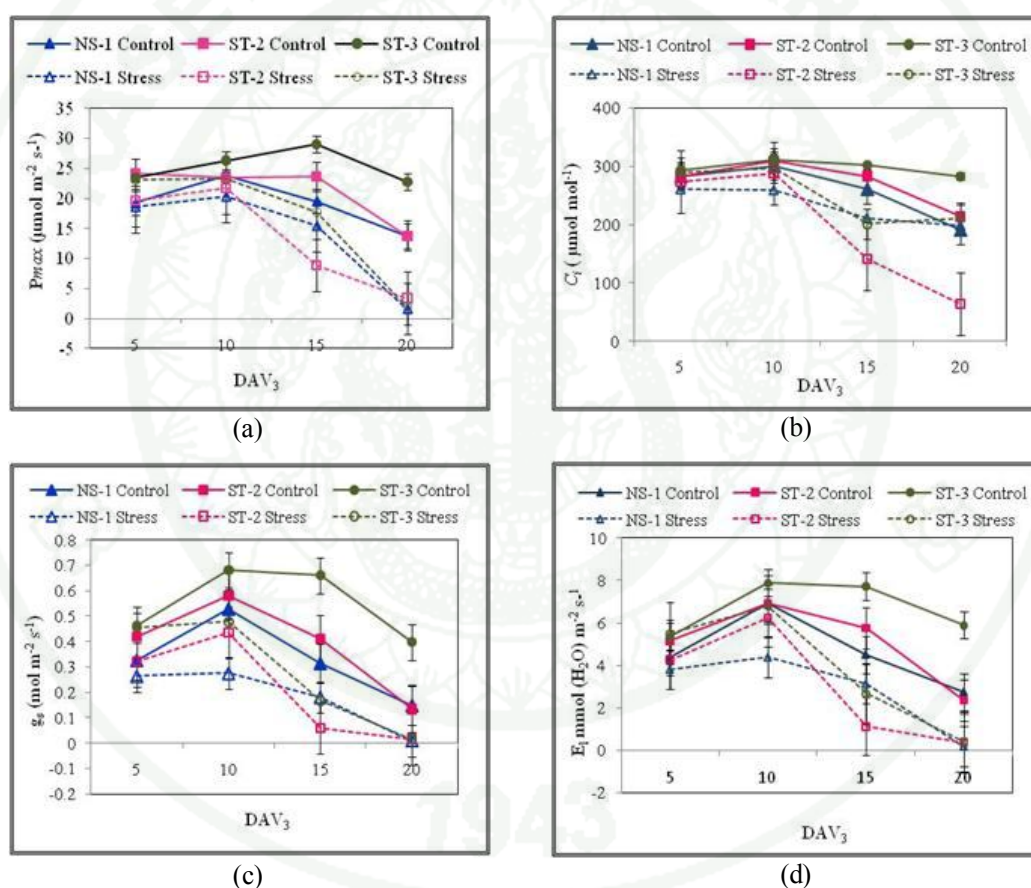
depletion, which was closed to the observed point at 20 DAV<sub>3</sub> in this study (Fig 10 and 11) whereas SM% remained about 25% under progressive soil dry water regime. Reduction in those concentrations of photosynthetic pigments (chlorophylls and carotenoids) in leaf was caused with the production of reactive oxygen species (ROS) in the thylakoids (Niyogi, 1999; Reddy *et al.*, 2004) while plants were subjected to drought stress. ROS has ability to damage greatly carotenoids which plays important role in anti-oxidant defense system to protect photosynthetic pigments (chlorophylls). As a consequence of reductions in anti-oxidants defense system lead to more suffering of oxidative damage (toxic elements production process in photosynthesis phenomenon), leading damage to photosynthesis pigments and reduced its photochemical reactions. On the plant nutrition aspect, under water deficit condition, minerals uptake were decreased in association with transpiration. Possibly, Mn and Mg ions uptake reduced which are essential for photosynthetic pigment synthesis. In addition, as a consequence of reduction in biosynthesis of various amino acids for photosynthetic pigments may lead insufficient replenishment to those pigments (Taiz and Zeiger, 2006; Sinha, 2005).

#### 4. Gas exchange and chlorophyll fluorescence

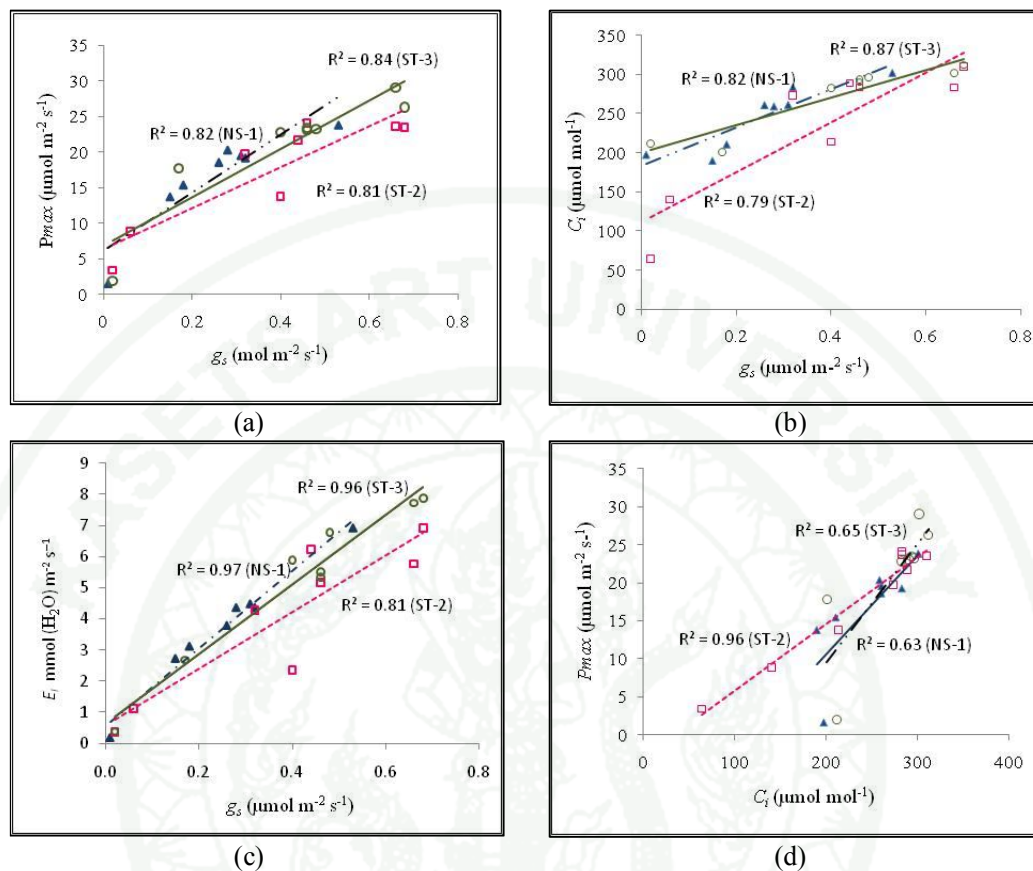
Photosynthesis capacities ( $P_{max}$ ) of plants under progressive soil dry water regime was not significantly different from control plants until 5 days after imposing stress, then had declined significantly at 10 DAV<sub>3</sub> and almost ceased at the end of dry down period. Similarly, stomata conductance ( $g_s$ ), transpiration rate ( $E_t$ ) and intercellular CO<sub>2</sub> ( $C_i$ ) were also significantly reduced under stressed (Fig 12).

Among the tested soybean cultivars, ST-3 had more  $P_{max}$  and maintained greater  $g_s$ ,  $C_i$  and  $E_t$  throughout observed period under both water regimes compared to the other cultivars. Furthermore, ST-3 maintained better in stability of its osmotic potential under both water regimes (Fig 10d). The results of changes in  $P_{max}$  and  $g_s$  of each cultivar (Fig 12) coincided with remaining soil moisture content and RWC as well (Fig 10a, and 10c).

As a result of greater in  $g_s$ , increased in  $E_l$ ,  $C_i$  and  $P_{max}$  under both water regimes. Therefore, it was obvious and well documented that photosynthesis was mainly governed by  $g_s$ . (Allen *et al.*, 1994; Ohashi *et al.*, 2006) and consequently effected on other photosynthetic process such as photochemical reactions, carboxylation (Lu *et al.*, 2009) efficiencies, intrinsic water use efficiencies were common in  $C_3$  plant species (Medrano *et al.*, 2002). Similarly,  $g_s$  showed high correlation with those photosynthesis parameters in all cultivars in this study (Fig 13).

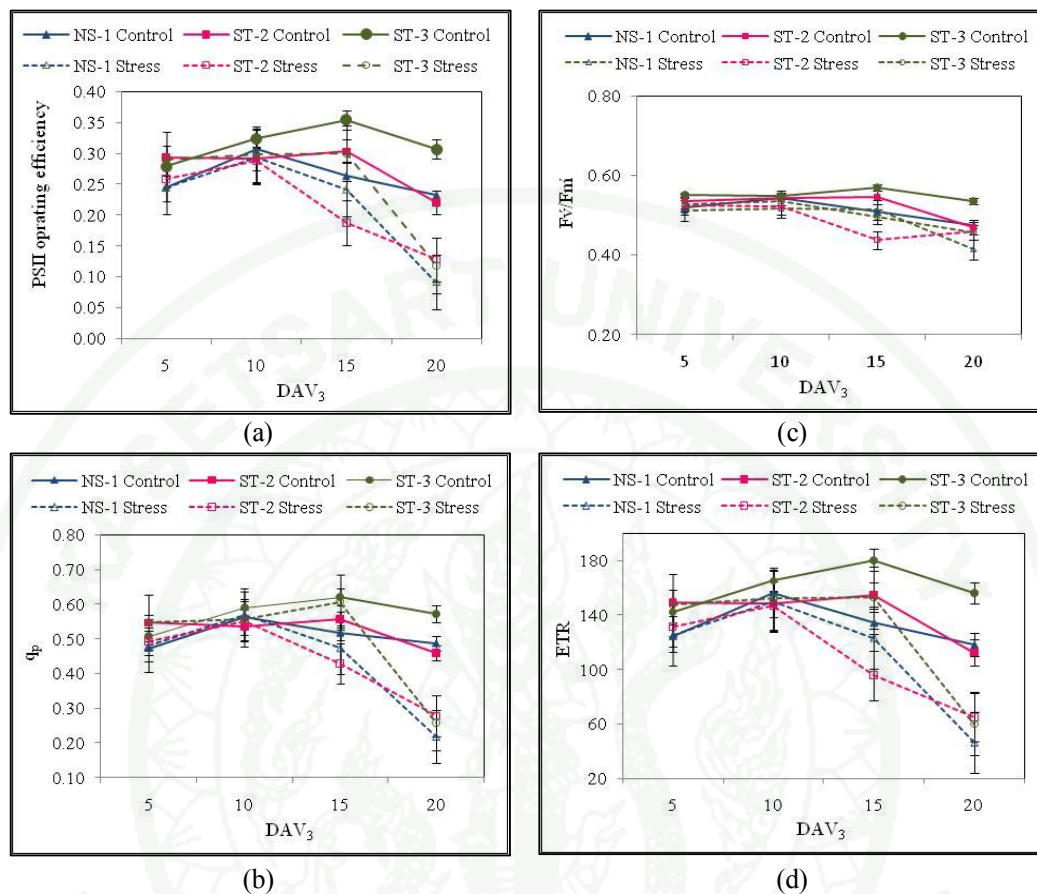


**Figure 12** (a) Photosynthesis capacity ( $P_{max}$ ); (b) stomata conductance ( $g_s$ ); (c) intercellular CO<sub>2</sub> ( $C_i$ ) and (d) transpiration rate ( $E_l$ ) in the leaves the soybean cultivars under watered (control) and progressive soil drying (Stress) water regimes at observed times. Bars indicate s.e (n=3).



**Figure 13** Relationship of stomata conductance ( $g_s$ ) with (a) photosynthesis capacity ( $P_{max}$ ); (b) transpiration rate in leaf ( $E_t$ ) and (c) inter-cellular  $\text{CO}_2$  concentration ( $C_i$ ) of leaves of the soybean cultivars and relation between  $P_{max}$  and  $C_i$  (d) under watered (Control) and progressive soil dry (Stress) water regimes. Bars indicate s.e ( $n=3$ ).

In this study, all observed chlorophyll fluorescence parameters did not significant between two water regimes, except maximum PSII efficiency ( $F_v/F_m'$ ) ( $p < 0.05$ ) at 10 DAV<sub>3</sub> (Fig 14). However,  $P_{max}$  decreased significantly ( $p < 0.05$ ) at 10 DAV<sub>3</sub> (Fig 12) while  $\text{Chl}_t$  (Fig 11) and SPAD (Table 12) were not significant between two water regimes.



**Figure 14** Photochemical activities ; (a) operating efficiency ( $F_q'/F_m'$ ); (b) PSII trapping efficiency ( $F_v'/F_m'$ ); (c) photochemical quenching ( $q_p$ ) and (d) relative electron transport rate (ETR) in the second fully expanded leaves of the cultivars under Controlled (Control) and progressive soil drying (Stress) water regimes at observed times. Bars indicate s.e (n=3).

Decreased in PSII maximum efficiency ( $F_v'/F_m'$ ) described the magnitude of light absorbed by chlorophyll was used in photochemistry of PSII decreased. Meanwhile the key photosynthetic enzyme Rubisco activity decreased by stomata controlled gas exchange for CO<sub>2</sub> assimilation (Parry *et al.*, 2002; Flexas *et al.*, 2006; Dias and Brüggemann, 2010) and energy demand (ATP and NADPH) for CO<sub>2</sub> assimilation was reduced, and consequently reduced relative energy (electron) transfer to PSI. However, there was not significant declined in ETR at 10 DAV<sub>3</sub> in this study indicated such magnitude of energy (electron) was utilized in Mehler –peroxidase

reactions and chloroplast metabolism (Fig 5), i.e, chlorophyll repairment-mechanism (Shinha 2004; Taiz and Zeiger, 2006). Biehler and Fock (1996) reported that 29.1% of the photosynthetic electrons consumed in the Mehler-peroxidase reaction and 18.4% drive the photorespiratory pathway in wheat (C<sub>3</sub> plant) under 14 days stress which at leaf water potential of -2.6 MPa.

All observed chlorophyll fluorescence parameters had declined significantly under prolong soil drying since 15 DAV<sub>3</sub> (Fig 14) which could explain to declining in overall photosynthesis that affected by water deficit in photochemical terms. As mentioned above, drastic reduced in photosynthesis under progressive soil dry water regime indicated reduction of Rubisco activity in impaired carbon metabolism, leading to the over accumulation of ATP and NADPH, then reducing the photosynthetic electron transport, resulting the over reduction of Q<sub>A</sub>, which all lead to the reduction of q<sub>p</sub> and operating efficiency of PSII (F<sub>q</sub>'/F<sub>m</sub>'), and dramatically reduced in PSII maximum efficiency (F<sub>v</sub>'/F<sub>m</sub>') (Melis, 1999). In addition, excess fluorescence or energy damaged to PSII itself and lead to photoinhibition (Taiz and Zeiger, 2006). On the other hand, excess fluorescence damaged to antioxidant system resulted as reduction photosynthetic pigments composition under sever water stress that showed in results of this study (Fig 11 and Table 13). There were no genotypic difference and interaction with water regimes in the all observed fluorescence parameters among the soybean cultivars.

##### 5. Plant growth and yield

Reduction in fresh and dry biomass (DM) under drought stress is a common adverse effect. Greater plant fresh and dry weights under water limited conditions are desirable characters to obtain better yield. Therefore, less in reduction of SDM and seed yield under drought stress being considered as a evaluation criterion for drought tolerance. The results in this study, SDM significantly declined ( $p < 0.0001$ ) at 15 DAV<sub>3</sub>.under progressive soil dry water regimes (Table 14).

**Table 14** Shoot dry matter ( $\text{g plant}^{-1}$ ) of the soybean cultivars (Cr) under watered (Control) and progressive soil drying (Stress) water regimes (W) at observed time.

Water regimes	Cultivar	Observed time				
		0 DAV <sub>3</sub>	5 DAV <sub>3</sub>	10 DAV <sub>3</sub>	15 DAV <sub>3</sub>	20 DAV <sub>3</sub>
Control	NS-1	0.82 <sup>ab</sup>	1.23 <sup>ab</sup>	1.61 <sup>b</sup>	2.75 <sup>a</sup>	4.09 <sup>b</sup>
	ST-2	0.93 <sup>a</sup>	1.39 <sup>a</sup>	2.15 <sup>a</sup>	2.81 <sup>a</sup>	4.71 <sup>a</sup>
	ST-3	0.76 <sup>b</sup>	1.05 <sup>b</sup>	1.69 <sup>b</sup>	2.93 <sup>a</sup>	3.87 <sup>b</sup>
Mean of control		0.84	1.23	1.81	2.85	4.22
Stress	NS-1	0.91 <sup>a</sup>	1.44 <sup>a</sup>	1.54 <sup>b</sup>	1.97 <sup>b</sup>	2.73 <sup>a</sup>
	ST-2	0.90 <sup>a</sup>	1.42 <sup>a</sup>	2.22 <sup>a</sup>	2.29 <sup>a</sup>	2.66 <sup>b</sup>
	ST-3	0.75 <sup>b</sup>	1.06 <sup>b</sup>	1.69 <sup>b</sup>	2.03 <sup>ab</sup>	2.70 <sup>a</sup>
Mean of stress		0.85	1.31	1.82	2.09	2.67
LSD <sub>0.05</sub> (W)		0.06	0.12	0.17	0.17	0.17
LSD <sub>0.05</sub> (Cr)		0.07	0.14	0.20	0.21	0.21
LSD <sub>0.05</sub> (Cr $\times$ W)		0.11	0.20	0.29	0.30	0.30
CV %		7.0	8.7	8.8	6.7	4.7

In a column, means followed by the same letter are not significant different at LSD<sub>0.05</sub>.

Tested cultivars showed genotypic difference in SDM accumulation under both water regimes (Table 14). ST-3 maintained its 69.98% of SDM of control as the highest at the end of dry down period and 55.82% of seed yield of control water regime (Table 15) as well. Seeds  $\text{plant}^{-1}$  and seed yield ( $\text{g plant}^{-1}$ ) was significantly reduced due to the effect of imposed water stress as results of decreased in pods, and number of seeds  $\text{pod}^{-1}$ , despite significant increased in 100 seed weight.

**Table 15** Seed yield (g plant<sup>-1</sup>) and yield components of the soybean cultivars (Cr) under watered (Control) and progressive soil drying (Stress) water regimes (W) at observed time.

Water regime	Cultivar	Number of pod	Seeds pod <sup>-1</sup>	100 seed weight(g)	Seed yield (g plant <sup>-1</sup> )
Control	NS-1	13.89	1.67	14.41	3.47
	ST-2	15.67	2.26	13.27	3.95
	ST-3	7.5	1.78	11.24	2.06
Mean of control		12.35	1.90	12.98	3.16
Stress	NS-1	6.17	0.96	14.54	1.91
	ST-2	6.67	0.89	14.85	1.74
	ST-3	5.00	0.69	11.24	1.15
Mean of stress		5.94	0.83	13.76	1.60
LSD <sub>0.05</sub> (W)		0.97	0.29	0.94	0.29
LSD <sub>0.05</sub> (Cr)		1.19	0.36	1.15	0.36
LSD <sub>0.05</sub> (CrxW)		1.68	0.51	1.62	0.50
CV %		10.6	20.5	5.0	9.4

In a column, means followed by the same letter are not significant different at LSD<sub>0.05</sub>

#### 6. Water Use Efficiency (WUE)

Stomata are very sensitive, responding to environmental changes that effected on gas exchange and transpiration and leading to variation in WUE. Therefore, it was suggested to approach with intrinsic water used efficiency (WUE<sub>in</sub>) which defined as the ratio of net CO<sub>2</sub> assimilation rate (A) and stomata conductance (g<sub>s</sub>), while interested in improving WUE, for example through breeding (Jones, 2004). Evidences pointed out that WUE<sub>in</sub> is rather constant over quite range of g<sub>s</sub> (Chaves *et al.*, 2004).

As a result of significant decrease in g<sub>s</sub> (Fig 12), WUE<sub>in</sub> had increased significantly since 10 DAV3 (Table 16). As usual, WUE<sub>in</sub> was greater in stress than

control leaves along with dry down period. Genotypic difference was only found at 10 DAV<sub>3</sub> under progressive soil dry water regime; NS-1 showed greater in WUE<sub>in</sub>, as a result of the lowest g<sub>s</sub>.(Fig 12).

**Table 16** WUE<sub>in</sub> of the soybean cultivars (Cr) under watered (Control) and progressive soil drying (Stress) water regimes (W) at observed time.

Water regime	cultivar	Observed time			
		5 DAV <sub>3</sub>	10 DAV <sub>3</sub>	15 DAV <sub>3</sub>	20 DAV <sub>3</sub>
Control	NS-1	59.5 <sup>a</sup>	46.4 <sup>a</sup>	73.5 <sup>a</sup>	119.5 <sup>a</sup>
	ST-2	57.8 <sup>a</sup>	41.6 <sup>a</sup>	57.3 <sup>a</sup>	105.3 <sup>a</sup>
	ST-3	52.1 <sup>a</sup>	39.3 <sup>a</sup>	44.2 <sup>a</sup>	58.2 <sup>a</sup>
Mean of control		56.5	42.5	58.5	94.3
Stress	NS-1	73.9 <sup>a</sup>	74.2 <sup>a</sup>	106.5 <sup>a</sup>	118.0 <sup>a</sup>
	ST-2	65.8 <sup>a</sup>	54.8 <sup>b</sup>	153.5 <sup>a</sup>	202.8 <sup>a</sup>
	ST-3	54.3 <sup>a</sup>	49.5 <sup>b</sup>	112.1 <sup>a</sup>	109.7 <sup>a</sup>
Mean of stress		64.7	59.5	124.1	143.5
LSD <sub>0.05</sub> (Cr)		16.1	13.1	33.6	118.5
LSD <sub>0.05</sub> (W)		13.1	10.7	27.4	96.8
LSD <sub>0.05</sub> (CrxW)		22.7	18.5	47.5	167.6
CV%		20.6	20.0	28.6	77.5

In a column, means followed by the same letter are not significant different at LSD<sub>0.05</sub>

As results of stomata closure under water deficits, decreased net transpiration of the plant (Fig 10 and 12) therefore increased in plant water use efficiency (Table 17). WUE<sub>plant</sub> of the soybean cultivars showed similarly as WUE<sub>in</sub>. Liu *et al.*, (2003) reported that WUE at both single leaf and whole plant levels was improved at mild water deficit and could improve plant yields which was closed to the point of 10 DAV<sub>3</sub> in this study whereas all the cultivars increased their shoot dry matter (Table 14) with greater water use efficiencies (Table 16 and 17).

**Table 17** WUE<sub>p</sub> of the soybean cultivars (Cr) under watered (Control) and progressive soil drying (Stress) water regimes (W) at observed time interval.

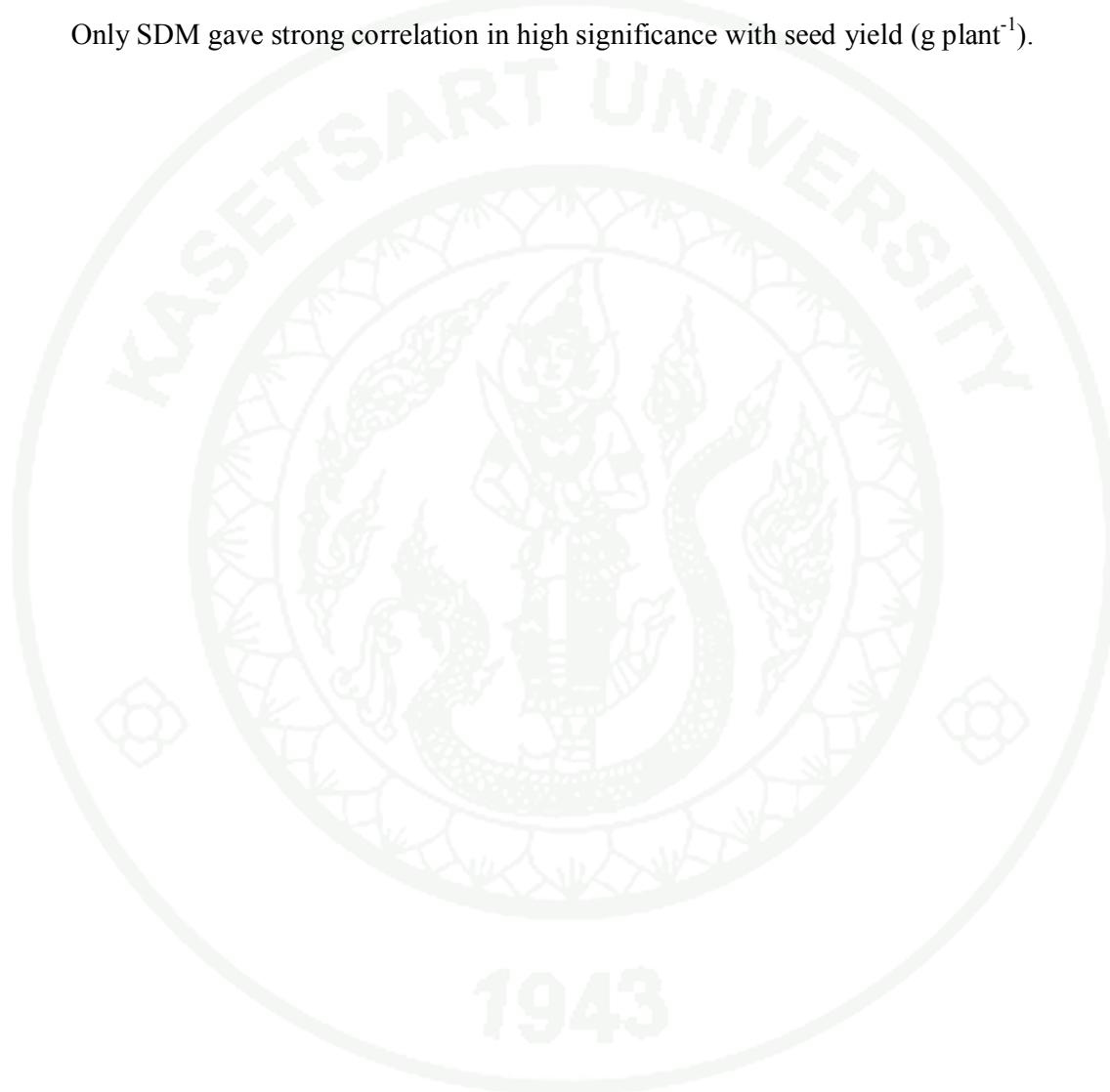
Water regimes	Cultivar	Observed time				
		0-5 DAV <sub>3</sub>	6-10 DAV <sub>3</sub>	11-15 DAV <sub>3</sub>	16-20 DAV <sub>3</sub>	0-20 DAV <sub>3</sub>
Control	NS-1	0.84 <sup>a</sup>	0.70 <sup>a</sup>	1.54 <sup>a</sup>	2.00 <sup>a</sup>	1.31 <sup>a</sup>
	ST-2	0.86 <sup>a</sup>	0.98 <sup>a</sup>	0.91 <sup>a</sup>	3.32 <sup>a</sup>	1.44 <sup>a</sup>
	ST-3	0.62 <sup>a</sup>	0.96 <sup>a</sup>	1.77 <sup>a</sup>	1.57 <sup>a</sup>	1.27 <sup>a</sup>
Mean of control		0.77	0.88	1.41	2.30	1.34
Stress	NS-1	0.93 <sup>a</sup>	0.26 <sup>a</sup>	1.29 <sup>a</sup>	4.96 <sup>a</sup>	1.17 <sup>a</sup>
	ST-2	1.21 <sup>a</sup>	1.53 <sup>a</sup>	0.17 <sup>a</sup>	2.45 <sup>b</sup>	1.15 <sup>a</sup>
	ST-3	0.79 <sup>a</sup>	1.20 <sup>a</sup>	0.77 <sup>b</sup>	4.28 <sup>ab</sup>	1.34 <sup>a</sup>
Mean of stress		0.98	0.99	0.74	3.90	1.22
LSD <sub>0.05</sub> (W)		0.31	0.46	0.61	1.39	0.14
LSD <sub>0.05</sub> (Cr)		0.37	0.59	0.75	1.70	0.17
LSD <sub>0.05</sub> (CrxW)		0.53	0.79	1.10	2.40	0.24
CV %		33	46	54	42	10.3

In a column, means followed by the same letter are not significant different at LSD<sub>0.05</sub>

### 7. Relations between SLW and other observed physiological parameters

Many mechanisms are underling in plant response to drought stress (Menavavalan *et al.*, 2009), therefore understanding to their relationship may help to select proper selection criteria for screening in drought tolerant soybean. In this study, SLW was aimed to use as a key selection criteria. Degree of correlation among observed parameters were varied with crop age. Greater relationships were observed at late reproductive stages than early. Significant and strong correlation between SLW, SPAD and other physiological and photo-chemical parameters were found at 20 DAV<sub>3</sub>, except with SLA (Table 18). Despite there was significant correlation between

SLW and seeds plant<sup>-1</sup>, no significant correlation with seed yield (gm plant<sup>-1</sup>), because correlation with seed yield (g plant<sup>-1</sup>) was affected by genotypic variation in seed weight of tested cultivars (Table 15) and increased in seed weight of stressed plants due to low competition for photo-synthates among seed sinks (Fellow *et al.*, 1987). Only SDM gave strong correlation in high significance with seed yield (g plant<sup>-1</sup>).



**Table 18** Simple correlation between specific leaf weight (SLW) and other observed parameter at 20 DAV<sub>3</sub> (R<sub>5</sub> stage).

	SLA	SPAD	Chl <sub>t</sub>	Pmax	g <sub>s</sub>	F <sub>q</sub> '/F <sub>m</sub> '	F <sub>v</sub> '/F <sub>m</sub> '	q <sub>p</sub>	WUE <sub>in</sub>	SDM	# Sd	SdY(g)
SLW	-0.23 <sup>ns</sup>	0.90 <sup>**</sup>	0.81 <sup>**</sup>	0.80 <sup>**</sup>	0.77 <sup>**</sup>	0.81 <sup>**</sup>	0.58 <sup>*</sup>	0.82 <sup>**</sup>	-0.60 <sup>**</sup>	0.64 <sup>**</sup>	0.47 <sup>*</sup>	0.32 <sup>ns</sup>
SLA		0.26 <sup>ns</sup>	-0.54 <sup>*</sup>	-0.10 <sup>ns</sup>	-0.08 <sup>ns</sup>	-0.17 <sup>ns</sup>	-0.37 <sup>ns</sup>	-0.12 <sup>ns</sup>	-0.06 <sup>ns</sup>	0.12 <sup>ns</sup>	0.35 <sup>ns</sup>	0.43 <sup>ns</sup>
SPAD			0.79 <sup>**</sup>	0.71 <sup>**</sup>	0.60 <sup>**</sup>	0.74 <sup>**</sup>	0.49 <sup>*</sup>	0.77 <sup>**</sup>	-0.47 <sup>*</sup>	0.78 <sup>**</sup>	0.65 <sup>**</sup>	0.52 <sup>*</sup>
Chl <sub>t</sub>				0.52 <sup>*</sup>	0.49 <sup>*</sup>	0.56 <sup>*</sup>	0.49 <sup>*</sup>	0.55 <sup>*</sup>	-0.49 <sup>*</sup>	-0.50 <sup>ns</sup>	0.27 <sup>*</sup>	0.11 <sup>**</sup>
Pmax					0.94 <sup>**</sup>	0.98 <sup>**</sup>	0.83 <sup>**</sup>	0.96 <sup>**</sup>	-0.46 <sup>*</sup>	0.71 <sup>**</sup>	0.58 <sup>*</sup>	0.43 <sup>ns</sup>
g <sub>s</sub>						0.89 <sup>**</sup>	0.77 <sup>**</sup>	0.86 <sup>**</sup>	0.57 <sup>*</sup>	0.53 <sup>*</sup>	0.40 <sup>ns</sup>	0.21 <sup>ns</sup>
F <sub>q</sub> '/F <sub>m</sub> '							0.84 <sup>**</sup>	0.99 <sup>**</sup>	-0.45 <sup>ns</sup>	0.72 <sup>**</sup>	0.57 <sup>*</sup>	0.43 <sup>ns</sup>
F <sub>v</sub> '/F <sub>m</sub> '								0.76 <sup>**</sup>	-0.27 <sup>ns</sup>	0.40 <sup>ns</sup>	0.21 <sup>ns</sup>	0.06 <sup>ns</sup>
q <sub>p</sub>									0.45 <sup>ns</sup>	0.78 <sup>**</sup>	0.65 <sup>**</sup>	0.53 <sup>*</sup>
WUE <sub>in</sub>										-0.41 <sup>ns</sup>	-0.36 <sup>ns</sup>	-0.19 <sup>ns</sup>
SDM											0.94 <sup>**</sup>	0.88 <sup>**</sup>
# Sd												0.97 <sup>**</sup>

SLA: specific leaf area, SLW: specific leaf weight, Ch<sub>t</sub>: total chlorophyll content, Pmax: photosynthetic capacity, g<sub>s</sub>: stomata conductance, F<sub>q</sub>'/F<sub>m</sub>': efficiency of open PSII centers, F<sub>v</sub>'/F<sub>m</sub>': the quantum yield of open PSII centers under irradiation, q<sub>p</sub>: photochemical quenching, ETR: relative electron transport rate, WUE<sub>in</sub>: intrinsic water use efficiency, SDM: shoot dry matter, #sd: number of seeds plant<sup>-1</sup>, SdY: seed yield (g plant<sup>-1</sup>) ns : not significant \* : significant at p≤ 0.05, \*\* and significant at p≤ 0.01, n=18

### Experiment III

#### **Nodule Growth and Nitrogen Fixation of Selected Soybean Cultivars under Different Soil Water Regimes**

In this experiment, three of the seven cultivars from the first experiment; NS-1, ST-2 and ST-3 were selected to evaluate their nodulation and N<sub>2</sub> fixation capacity under different soil water regimes. During sampling period, average of day and night temperature were 33.6° C (±2.1) and 24.1° C (±0.9), and day length was approximately 12.8 hr.

##### 1. Soil and plant water status

Control plants maintained soil moisture of 50.4% (±1.5) throughout the observed period. Under water stress conditions, the observed soil moisture percentage were 28.1.9 (±3.9), 14.2 (±0.8), 13.3(±0.8) at 7, 12, 15 DAV<sub>4</sub>, respectively. There were no significant differences among the cultivars in the remaining SM% at all observed times. As for plant water status, controlled plants maintained around 79.26% (±3.7) of relative water content (RWC) of the leaf throughout the observed period. RWC of the leaf under water stress was significantly decreased ( $p < 0.01$ ) with increasing soil drying and maintained at 51.6% (±4.2), 47.2% (±6.1), 43.3% (±6.6) at 7, 12 and 15 DAV<sub>4</sub>, respectively. However, there were no significant differences in RWC of the leaf of the tested cultivars at each observed time within the same water regime and no interaction between water regime and cultivar.

##### 2. Shoot and root growth

Shoot dry matter (SDM) increased with crop age under well watered regime. Under progressive soil drying regime, SDM significantly reduced ( $p < 0.01$ ) since 12 DAV<sub>4</sub> (Table 19). There were no significant different SDM among the cultivars under stress water regime except at 7 DAV<sub>4</sub>. SDM of most cultivars still

decreased after re-watered at 16 DAV<sub>4</sub> due to continued leaf senescence (Brevedan and Egli, 2003) and probably utilization of reserved food for their new growth (Fellow *et al.*, 1987).

**Table 19** Shoot dry matter (g plant<sup>-1</sup>) of the soybean cultivars (Cr) under watered (Control) and progressive soil drying (Stress) water regimes (W) at observed times.

Water regimes	Cultivar	Observed time				
		0 DAV <sub>4</sub>	7 DAV <sub>4</sub>	12 DAV <sub>4</sub>	15 DAV <sub>4</sub>	22DAV <sub>4</sub>
Control	NS-1	1.02 <sup>a</sup>	2.13 <sup>a</sup>	2.76 <sup>a</sup>	3.85 <sup>ab</sup>	6.32 <sup>a</sup>
	ST-2	0.75 <sup>b</sup>	1.82 <sup>ab</sup>	3.25 <sup>ab</sup>	4.52 <sup>a</sup>	6.75 <sup>a</sup>
	ST-3	0.62 <sup>b</sup>	1.55 <sup>b</sup>	2.59 <sup>b</sup>	3.50 <sup>b</sup>	5.16 <sup>b</sup>
Mean of control		0.79	1.83	2.86	3.96	6.07
Stress	NS-1	0.80 <sup>a</sup>	2.08 <sup>a</sup>	2.33 <sup>a</sup>	2.38 <sup>a</sup>	1.65 <sup>a</sup>
	ST-2	0.83 <sup>b</sup>	1.75 <sup>ab</sup>	2.22 <sup>a</sup>	2.41 <sup>a</sup>	1.69 <sup>a</sup>
	ST-3	0.60 <sup>b</sup>	1.32 <sup>b</sup>	1.84 <sup>a</sup>	2.01 <sup>a</sup>	1.77 <sup>a</sup>
Mean of stress		0.80	1.71	2.13	2.27	1.70
LSD <sub>0.05</sub> (W)		0.11	0.29	0.30	0.43	0.55
LSD <sub>0.05</sub> (Cr)		0.14	0.35	0.36	0.52	0.67
LSD <sub>0.05</sub> (Cr×W)		0.19	0.50	0.52	0.74	0.95
CV %		12.8	15.4	11.4	13.1	13.5

In a column, means followed by the same letter are not significant different at LSD<sub>0.05</sub>

Root growth had greater under water stress over control at 12 DAV<sub>4</sub> but not significant. ST-2 and ST-3 showed greater root growth than NS-1 at 12 DAV<sub>4</sub>. However, there was no significant among tested cultivars at the end of withholding water stress and at recovered after re-watering as well (Table 20).

**Table 20** Root dry matter ( $\text{g plant}^{-1}$ ) of the soybean cultivars (Cr) under watered (Control) and re-watered from 15 days progressive soil drying (Stress) water regimes (W) at observed times.

Water regimes	Cultivar	Observed time				
		0 DAV <sub>4</sub>	7 DAV <sub>4</sub>	12 DAV <sub>4</sub>	15 DAV <sub>4</sub>	22DAV <sub>4</sub>
Control	NS-1	0.28 <sup>a</sup>	0.32 <sup>a</sup>	0.33 <sup>c</sup>	0.43 <sup>c</sup>	0.56 <sup>b</sup>
	ST-2	0.20 <sup>a</sup>	0.32 <sup>a</sup>	0.49 <sup>a</sup>	0.52 <sup>a</sup>	0.77 <sup>a</sup>
	ST-3	0.23 <sup>a</sup>	0.27 <sup>a</sup>	0.36 <sup>b</sup>	0.48 <sup>b</sup>	0.56 <sup>b</sup>
Mean of control		0.24	0.30	0.39	0.48	0.63
Stress	NS-1	0.27 <sup>a</sup>	0.32 <sup>a</sup>	0.40 <sup>b</sup>	0.34 <sup>a</sup>	0.32 <sup>a</sup>
	ST-2	0.25 <sup>a</sup>	0.28 <sup>a</sup>	0.42 <sup>a</sup>	0.40 <sup>a</sup>	0.34 <sup>a</sup>
	ST-3	0.26 <sup>a</sup>	0.27 <sup>a</sup>	0.42 <sup>a</sup>	0.41 <sup>a</sup>	0.32 <sup>a</sup>
Mean of stress		0.26	0.29	0.41	0.38	0.33
LSD <sub>0.05</sub> (W)		0.05	0.07	0.06	0.05	0.07
LSD <sub>0.05</sub> (Cr)		0.06	0.09	0.07	0.06	0.08
LSD <sub>0.05</sub> (CrxW)		0.09	0.13	0.11	0.08	0.11
CV %		20.0	23.5	14.4	11.0	13.1

In a column, means followed by the same letter are not significant different at LSD<sub>0.05</sub>

Nodule dry matter increased over time under control water regime. Under withholding water stress, nodule was suppressed and significantly decreased ( $p < 0.01$ ) since 7 DAV<sub>4</sub> (Table 21). Because, oxygen diffusion and sucrose substrate were limited under water deficits and consequently affected on nodule growth and functions (Cordon *et al.*, 1997; Gálevz *et al.*, 2005).

**Table 21** Total nodule dry weight, total nodule number and individual nodule dry weight of soybean cultivars (Cr) at observed time under well watered (Control) and re-watered from 15 days progressive soil drying (Stress) water regimes (W).

Water regime	Cultivar	Observed time				
		0 DAV <sub>4</sub>	7 DAV <sub>4</sub>	12 DAV <sub>4</sub>	15 DAV <sub>4</sub>	22 DAV <sub>4</sub>
<u>Total nodule dry weight (mg plant<sup>-1</sup>)</u>						
Control	NS-1	393 <sup>a</sup>	435 <sup>a</sup>	464 <sup>a</sup>	519 <sup>a</sup>	629 <sup>a</sup>
	ST-2	358 <sup>ab</sup>	402 <sup>b</sup>	438 <sup>a</sup>	517 <sup>a</sup>	600 <sup>ab</sup>
	ST-3	353 <sup>b</sup>	388 <sup>b</sup>	439 <sup>a</sup>	481 <sup>a</sup>	586 <sup>a</sup>
Mean of control		368	408	447	506	605
Stress	NS-1	383 <sup>a</sup>	387 <sup>a</sup>	375 <sup>a</sup>	335 <sup>a</sup>	333 <sup>a</sup>
	ST-2	367 <sup>a</sup>	386 <sup>a</sup>	395 <sup>a</sup>	342 <sup>a</sup>	323 <sup>a</sup>
	ST-3	368 <sup>a</sup>	382 <sup>a</sup>	352 <sup>a</sup>	345 <sup>a</sup>	326 <sup>a</sup>
Mean of stress		372	385	374	341	327
LSD <sub>0.05</sub> W		11.25	15.13	29.15	22.79	15.37
LSD <sub>0.05</sub> W x Cr		19.5	26.2	50.5	39.5	26.6
CV%		2.9	3.6	6.8	5.1	3.1
<u>Total number of nodules plant<sup>-1</sup></u>						
Control	NS-1	52.78 <sup>a</sup>	62.11 <sup>a</sup>	65.22 <sup>a</sup>	71.22 <sup>b</sup>	93.56 <sup>ab</sup>
	ST-2	33.67 <sup>ab</sup>	51.67 <sup>ab</sup>	65.78 <sup>a</sup>	80.55 <sup>a</sup>	119.89 <sup>a</sup>
	ST-3	28.22 <sup>b</sup>	42.56 <sup>b</sup>	51.33 <sup>a</sup>	55.22 <sup>c</sup>	79.22 <sup>b</sup>
Mean of control		38.22	52.11	60.78	68.96	97.56
Stress	NS-1	56.78 <sup>a</sup>	45.79 <sup>a</sup>	43.15 <sup>a</sup>	23.78	27.11 <sup>a</sup>
	ST-2	33.11 <sup>b</sup>	42.78 <sup>a</sup>	30.11 <sup>ab</sup>	21.33 <sup>a</sup>	18.67 <sup>a</sup>
	ST-3	34.00 <sup>b</sup>	38.78 <sup>a</sup>	22.78 <sup>b</sup>	18.00 <sup>a</sup>	19.33 <sup>a</sup>
Mean of stress		41.30	42.11	32.01	21.04 <sup>a</sup>	21.70 <sup>a</sup>
LSD <sub>0.05</sub> W		9.24	10.39	10.25	5.43	21.43
LSD <sub>0.05</sub> W x Cr		16.00	18.00	17.76	9.40	37.12
CV%		22.10	21.0	21.0	11.5	34.2
<u>Individual nodule dry weight (mg nodule<sup>-1</sup>)</u>						
Control	NS-1	7.46 <sup>a</sup>	7.19 <sup>a</sup>	7.19 <sup>a</sup>	7.30 <sup>a</sup>	6.78 <sup>a</sup>
	ST-2	11.45 <sup>ab</sup>	7.92 <sup>a</sup>	6.80 <sup>a</sup>	6.46 <sup>a</sup>	5.39 <sup>a</sup>
	ST-3	12.80 <sup>a</sup>	9.19 <sup>a</sup>	8.58 <sup>a</sup>	8.79 <sup>a</sup>	7.49 <sup>a</sup>
Mean of control		9.67	8.10	7.52	7.52	6.55
Stress	NS-1	6.92 <sup>b</sup>	8.78 <sup>a</sup>	9.46 <sup>b</sup>	14.25 <sup>b</sup>	13.46 <sup>a</sup>
	ST-2	12.02 <sup>a</sup>	9.83 <sup>a</sup>	14.48 <sup>ab</sup>	16.67 <sup>a</sup>	23.32 <sup>a</sup>
	ST-3	10.99 <sup>ab</sup>	10.07 <sup>a</sup>	20.54 <sup>a</sup>	21.51 <sup>a</sup>	17.54 <sup>a</sup>
Mean of stress		10.88	9.56	14.83	17.25	18.1
LSD <sub>0.05</sub> W		2.40	2.11	4.45	3.59	7.49
LSD <sub>0.05</sub> W x Cr		4.17	3.66	7.71	6.23	12.88
CV%		22.3	22.8	37.9	27.4	57.4

In a column, means followed by the same letter are not significant different at LSD<sub>0.05</sub>

There were no interaction between cultivars and water regimes throughout the observed times. Genotypic difference was found only under control water regime in early observations and last observation (Table 21). Both total nodule number (TNN) and individual nodule dry weight (INDW) had significantly declined at 12 DAV<sub>4</sub>. Differences in TNN among the cultivars were frequently found under control water regime. But it was found only at 12 DAV<sub>4</sub> under stressed water regime. Inverse relationship existed between TNN and INDW under both water regimes. ST-2 had greatest total nodule number (TNN), therefore lowest in INDW and ST-3 showed reverse manner under control water regime. NS-1 had greatest TNN and lowest INDW under stressed water regime. However, there were no differences in all nodule parameters of the cultivars under stress water regime, except at V<sub>4</sub> stage. NS-1 had greatest NDW as result of highest TNN in both growing season compared among same three cultivars. Sinclair *et al.* (1991) reported genotypic variation in NDW and TNN were consistence across years and locations. Fellow *et al.* (1987) reported that nodule activity fully recovered within 2 days after re-watering from withholding water stress imposed for 7 days at V<sub>7</sub> stage. However, in this study had not well recovered in all cultivars after re-watering, probably due to longer dry down period and less allocation of photo-assimilates to nodules when plants were in seed filling stage (Gomes and Sodek, 1987).

### 3. SPADD reading

Chlorophyll meter (SPAD: *M-502*) has been widely use to access primarily to detect chlorophyll content. Significant correlations between photosynthesis and leaf N content have been reported for a large number of species, including soybean (Boote *et al.*, 1978; Buttery and Buzzel, 1988). A positive correlation between leaf N or N fertilization rate and chlorophyll content is well documented for a large number of crop species and has been investigated for rapid N status determination using Chl meters in most major crops including soybean (Ma *et al.*, 1995). However, Fritschi and Ray (2007) reported that although there were highly significant ( $p < 0.0001$ ) correlation between SPAD reading, not sufficient to predict leaf N content across a

large number of soybean germplasm. On the other hand, both leaf chlorophyll content and shoot total N had also positively correlated with SPAD readings in soybean (Mirza *et al.*, 2001). SPAD reading was applied to differentiate between promiscuous and non-promiscuous nodulation in soybean (Gwata *et al.*, 2004). They reported that leaf color score transformed from SAPD reading had positive significant ( $p < 0.01$ ) with NDW. In this study, SPAD reading had significantly declined ( $p < 0.01$ ) since 12 DAV<sub>4</sub> under soil dry water regime (15 DAV<sub>4</sub>). There was no genotypic difference of SPAD reading within same water regime (Table 22).

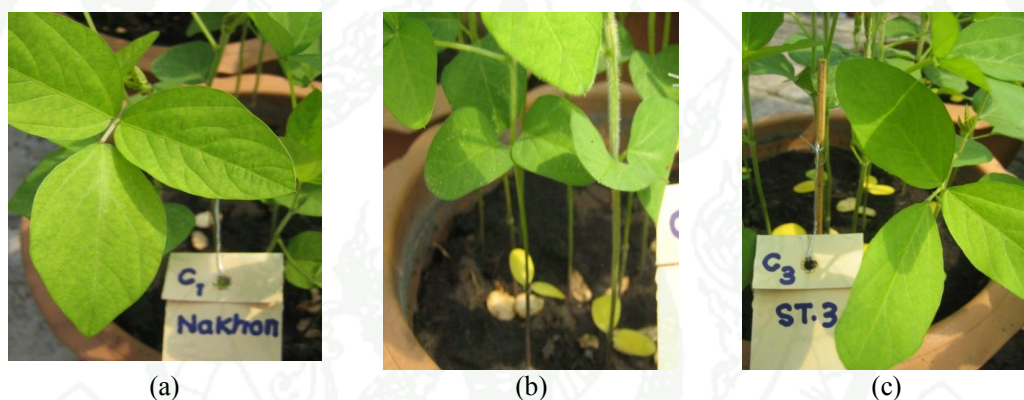
**Table 22** SPAD reading of the second most fully expanded leaf of soybean cultivars (Cr) at observed time under well watered (Control) and re-watered from 15 days progressive soil drying (Stress) water regimes (W).

Water regimes	Cultivar	Observed time				
		0 DAV <sub>4</sub>	7 DAV <sub>4</sub>	12 DAV <sub>4</sub>	15 DAV <sub>4</sub>	22DAV <sub>4</sub>
Control	NS-1	29.8 <sup>a</sup>	33.7 <sup>a</sup>	36.1 <sup>a</sup>	36.8 <sup>a</sup>	36.4 <sup>a</sup>
	ST-2	28.5 <sup>a</sup>	30.0 <sup>a</sup>	35.1 <sup>a</sup>	36.9 <sup>a</sup>	35.2 <sup>a</sup>
	ST-3	30.6 <sup>a</sup>	33.8 <sup>a</sup>	35.6 <sup>a</sup>	37.5 <sup>a</sup>	40.3 <sup>a</sup>
	Mean of control	29.6	32.5	35.6	37.1	36.7
Stress	NS-1	30.7 <sup>a</sup>	32.6 <sup>a</sup>	32.5 <sup>a</sup>	28.4 <sup>a</sup>	37.3 <sup>a</sup>
	ST-2	28.7 <sup>a</sup>	29.8 <sup>a</sup>	31.0 <sup>a</sup>	27.1 <sup>a</sup>	34.7 <sup>a</sup>
	ST-3	29.3 <sup>a</sup>	32.7 <sup>b</sup>	34.3 <sup>a</sup>	29.8 <sup>a</sup>	36.7 <sup>a</sup>
	Mean of stress	29.6	31.7	32.6	28.7	36.2
LSD <sub>0.05</sub> (W)		0.13	0.21	0.26	0.32	0.34
LSD <sub>0.05</sub> (WxCr)		2.3	3.7	4.5	5.6	5.9
CV %		4.2	6.3	7.3	9.4	8.9

In a column, means followed by the same letter are not significant different at LSD<sub>0.05</sub>

#### 4. Nitrogen fixation of the cultivars under different water regimes

As a result of recycled used soil, several soil washings, high seeding rate and a little delay in thinning, all cultivars showed temporary nitrogen symptoms with early yellowing in cotyledon and leaf chlorosis around V<sub>3</sub> stage (Fig 15), but disappeared later. Therefore, it was assumed that plants received nitrogen from mainly from N<sub>2</sub> fixation in this experiment.



**Figure 15** Temporary nitrogen deficiency symptom of early yellowing of cotyledon and leaf chlorosis on the leaf at V<sub>3</sub> growth stage of the cultivar (a) NS-1 (b) ST-2 and (c) ST-3.

N concentration in shoot biomass (N  $\mu\text{g}$  of  $\text{g}^{-1}$  shoot dry matter) had significantly declined under soil drying water regimes since 15 DAV<sub>4</sub> (Table 23). However, N<sub>2</sub> fixation ability of the tested cultivars were compared in terms of N concentration in the accumulated shoot biomass (King and Purcell, 2006) within the two shoot biomass harvests (7 and 15 DAV<sub>4</sub>).

**Table 23** Total N in the shoot biomass of soybean cultivars (Cr) at 7, 12, 15 and 22 DAV<sub>4</sub> under well watered (Control) and re-watered from 15 days progressive soil drying (Stress) water regimes (W).

Water regime	cultivar	Observed time			
		7 DAV <sub>4</sub>	12 DAV <sub>4</sub>	15 DAV <sub>4</sub>	22 DAV <sub>4</sub>
Control	NS-1	10.44 <sup>ab</sup>	10.04 <sup>a</sup>	12.17 <sup>a</sup>	13.51 <sup>a</sup>
	ST-2	10.03 <sup>b</sup>	10.86 <sup>a</sup>	10.77 <sup>b</sup>	10.84 <sup>b</sup>
	ST-3	10.76 <sup>a</sup>	10.80 <sup>a</sup>	12.11 <sup>ab</sup>	12.79 <sup>ab</sup>
Mean of control		10.74	10.90	11.69	12.38
Stress	NS-1	10.44 <sup>a</sup>	10.62 <sup>a</sup>	10.92 <sup>a</sup>	10.32 <sup>a</sup>
	ST-2	11.81 <sup>a</sup>	10.62 <sup>a</sup>	10.50 <sup>a</sup>	9.49 <sup>a</sup>
	ST-3	10.62	10.15 <sup>ab</sup>	11.22 <sup>a</sup>	9.37 <sup>a</sup>
Mean of stress		10.96	11.69	10.88	9.79
LSD <sub>0.05</sub> W		0.79	0.43	0.57	1.24
LSD <sub>0.05</sub> W x C <sub>1</sub>		1.37	0.75	0.99	2.15
CV%		6.9	3.9	4.8	10.7

In a column, means followed by the same letter are not significant different at LSD<sub>0.05</sub>

Both nitrogen and shoot biomass accumulation rates were significantly declined under stress water regime ( $p < 0.01$ ) compared to control water regime (Table 23). Nitrogen concentrations in accumulated shoot biomass of stressed plants were lower than controls in all cultivars which was similar with results of King and Purcell (2006).

The results were consistent with SPAD reading (Table 22). There were no interactions between cultivar and water stress at all observed parameters. King and Purcell (2006) suggested that shoot N concentration of well watered plants is more likely to a better indicator of genotypic response of shoot N concentration to drought water stress than shoot ureides of well watered plants.

**Table 24** Accumulation rates of Nitrogen and shoot biomass, Nitrogen concentration in accumulated shoot biomass between 7 and 15 DAV<sub>4</sub> of soybean cultivars (Cr) under well watered (Control) and progressive soil drying (Stress) water regimes (W).

Water regime	Cultivar	Accumulation rate		N concentration in accumulated shoot mass ( $\mu\text{gN g}^{-1}$ of SDM)
		N ( $\mu\text{gN plant}^{-1} \text{d}^{-1}$ )	Shoot biomass ( $\text{g plant}^{-1} \text{d}^{-1}$ )	
Control	NS-1	3.10 <sup>a</sup>	0.21 <sup>b</sup>	14.24 <sup>a</sup>
	ST-2	3.78 <sup>a</sup>	0.34 <sup>a</sup>	11.31 <sup>a</sup>
	ST-3	3.37 <sup>a</sup>	0.24 <sup>b</sup>	13.98 <sup>a</sup>
Mean of control		3.42	0.266	13.17
Stress	NS-1	0.53 <sup>a</sup>	0.04 <sup>b</sup>	14.00 <sup>a</sup>
	ST-2	0.54 <sup>a</sup>	0.08 <sup>a</sup>	7.33 <sup>a</sup>
	ST-3	0.76 <sup>a</sup>	0.08 <sup>a</sup>	9.23 <sup>a</sup>
Mean of stress		0.61	0.067	10.18
LSD <sub>0.05</sub> W		0.66	0.049	4.58
LSD <sub>0.05</sub> W x Cr		1.14	0.085	7.92
CV%		31.0	28.0	37.3

In a column, means followed by the same letter are not significant different at LSD<sub>0.05</sub>

In addition, dimensionless tolerant ratio of N fixation (Sincalir *et al.*, 2000) was also considered in cultivar comparison in this study. N<sub>2</sub> fixation tolerance ratio was also calculated by difference in N accumulated in the shoot between two biomass samples was divided by the difference in biomass between two harvests of sampling. Consequently, the calculated value was divided for each cultivar by the N concentration of particular cultivar of the first harvest. This calculation rested in a dimensionless tolerant ratio (Sinclair *et al.*, 2000), which in the most case had a value of less than one. This tolerance ratio was interpreted to reflect the ability of each cultivar to sustain N<sub>2</sub> fixation rate during the water deficit treatment so as to maintain the initial N concentration of the plant. A high value in this ratio was taken to indicate

a potential tolerance of N<sub>2</sub> fixation to soil drying. Based on N<sub>2</sub> fixation tolerance ratio, NS-1 was highest among tested cultivars (Table 25).

**Table 25** Difference of accumulated N in accumulated shoot biomass and shoot biomass between 7 and 15 DAV<sub>4</sub> and N<sub>2</sub> fixation tolerance ratio of soybean cultivars (Cr) under well watered (Control) and withholding water-stressed (Stress) water regimes (W).

Water regime	Cultivar	Difference in accumulated		Shoot N concentration at 7 DAV <sub>4</sub> (µgN g <sup>-1</sup> of SDM)	Tolerance ratio
		N (µgN plant <sup>-1</sup> )	Shoot biomass (g plant <sup>-1</sup> )		
Control	NS-1	24.78 <sup>a</sup>	1.72 <sup>b</sup>	10.44 <sup>a</sup>	0.94
	ST-2	30.26 <sup>a</sup>	2.70 <sup>a</sup>	10.62 <sup>a</sup>	0.55
	ST-3	26.95 <sup>a</sup>	1.96 <sup>ab</sup>	11.76 <sup>a</sup>	0.74
Mean of control		27.33	2.13	10.94	
Stress	NS-1	4.26 <sup>a</sup>	0.31 <sup>a</sup>	10.62 <sup>a</sup>	
	ST-2	4.29 <sup>a</sup>	0.66 <sup>a</sup>	12.23 <sup>a</sup>	
	ST-3	6.11 <sup>a</sup>	0.69 <sup>a</sup>	11.04 <sup>a</sup>	
Mean of stress		4.88	0.56	11.30	
LSD <sub>0.05</sub> W		5.25	0.40	0.99	
LSD <sub>0.05</sub> W x Cr		9.10	0.68	1.71	
CV%		31.0	28.0	8.4	

In a column, means followed by the same letter are not significant different at LSD<sub>0.05</sub>

Nitrogen concentration in shoot mass could be used to estimate N<sub>2</sub> fixation capacity and allowed to assess such a number of samples. However, by using acetylene reduction assay (ARA) is more precise to estimate N<sub>2</sub> fixation ability and widely accepted, because, acetylene strongly interacts with all forms of substrates for nitrogenase enzyme (Hardy *et al.*, 1968). In this study, N<sub>2</sub> fixation abilities of the selected cultivars were also evaluated with ARA. Results of ARA were presented in (Table 26).

**Table 26** ARA ( $\mu\text{mol C}_2\text{H}_4 \text{ hr}^{-1} \text{ plant}^{-1}$ ) of fresh nodulated roots of soybean cultivars (Cr) at 7, 12, 15 and 22 DAV<sub>4</sub> under well watered (Cont) and re-watered from 15 days progressive soil drying (Str) water regimes (W).

Cultivar	7 DAV <sub>4</sub>		12DAV <sub>4</sub>		15DAV <sub>4</sub>		22DAV <sub>4</sub>	
	Cont	Str	Cont	Str	Cont	Str	Cont	Str
NS-1	19.96 <sup>a</sup>	15.27 <sup>a</sup>	24.28 <sup>a</sup>	2.80 <sup>a</sup>	27.13 <sup>b</sup>	1.94 <sup>a</sup>	14.44 <sup>c</sup>	5.46 <sup>a</sup>
ST-2	15.24 <sup>b</sup>	13.69 <sup>ab</sup>	16.15 <sup>ab</sup>	2.10 <sup>a</sup>	31.49 <sup>ab</sup>	2.10 <sup>a</sup>	26.58 <sup>a</sup>	4.67 <sup>a</sup>
ST-3	14.21 <sup>b</sup>	12.67 <sup>b</sup>	13.30 <sup>b</sup>	2.06 <sup>a</sup>	37.87 <sup>a</sup>	2.37 <sup>a</sup>	22.85 <sup>b</sup>	4.76 <sup>a</sup>
Mean	16.47	13.88	17.91	2.31 <sup>a</sup>	32.17	2.14	21.29	4.96
LSD <sub>0.05</sub> W		2.14		1.78		3.17		3.02
LSD <sub>0.05</sub> W x Cr		3.71		3.08		5.49		5.23
CV%		13.5		16.7		17.6		21.9
Relative NA (%)								
NS-1		76.50		11.53		7.15		37.81
ST-2		89.83		13.00		6.67		17.57
ST-3		84.27		12.89		6.65		20.83

In a column, means followed by the same letter are not significant different at LSD<sub>0.05</sub>

Under control water regime, NA of nodulated root of all cultivars showed gradual increase with increasing nodule mass until 15 DAV<sub>4</sub>. But ARA declined at 22 DAV<sub>4</sub> (R<sub>5</sub> stage). This indicated limitation of substrates from photo-assimilates to nodule which caused the decline in NA under well-watered regimes (Gomes and Sodek, 1987) as similar to NDW. Under control water regime, NS-1 showed highest NA in early periods, but ST-3 become highest at the later period because NS-1 was relatively an early maturing cultivar.

Nitrogenase activity was also depressed under stressed water regime like nodule growth. NA under stressed water regime had significantly decreased ( $p < 0.05$ ) since 7 DAV<sub>4</sub> while plants maintained RWC in leaf around 53.07% ( $\pm 5.3$ ) whereas soil moisture remained at 28.1.9% ( $\pm 3.9$ ). However, at 7 DAV<sub>4</sub>, SDM had not significantly decreased yet under progressive soil dry water regime (Table 19). Regarding to the fact and the results of SDM and NA indicated that N<sub>2</sub> fixation was much affected earlier than shoot growth. Steerer (2003) reported that major decrease

in  $N_2$  fixation activity was due to lower demand for fixed N to support growth, not due to carbon supply to bacteroids. On the other hand, limitation of carbon supply to nodules involved in decline of  $N_2$  fixation under early drought had been reported (Gálevz *et al.*, 2005;Laudera *et al.*, 2007). Huang *et al* (1975) also reported that inhibition of shoot photosynthesis account for inhibition in  $N_2$  fixation in soybean at low water potential. In addition, according to Wang *et al.* (2006), soybean net photosynthesis dramatically declined after remaining soil moisture at 26% which was closed to the point at 7 DAV<sub>4</sub> in this study. Accumulated evidences suggested and pointed that declined in  $N_2$  fixation was associated with declined in photosynthesis under drought; therefore the two processes were inter-dependent. In addition, dehydration and tissue damage in nodule (Huang *et al.*, 1975; Albrecht *et al.*, 1984) lead to low  $O_2$  diffusion (Cordon *et al.*, 1997), dramatically decreased in sucrose synthase activities in nodule (González *et al.*, 1995), limitation of carbon flux and sucrose (Gálevz *et al.*, 2005), and feedback inhibition of N accumulation in leaves and nodule (Laudera *et al.*, 2007) have been reported for sensitivity of NA to moisture stress.

NA had severely declined about eight folds at 12 DAV<sub>4</sub> while plants maintained RWC in leaf around 48.50% ( $\pm 7.8$ ) and the soil moisture remained at 14.2% ( $\pm 0.8$ ). There was no significant difference among the cultivars at 12 DAV<sub>4</sub> under progressive soil dried water regime.

Regarding the relative NA of nodulated root of stress plants, it varied with intensity of stress along with crop development stage (Table 26) and nodule development (Table 21). At the end of soil drying period, NS-1 sustained NA (7.15%) than ST-2 (6.67%) and ST-3 (6.65%) which was similar to ranking in N concentration in accumulated shoot biomass and tolerance ratio. Interaction between water treatments and cultivars was not found at 7 DAV<sub>4</sub>, but it was found at the later observations due to ARA differences among cultivars under well watered regime. Regarding the results of N concentration in accumulated shoot biomass (Table 24), tolerance ratio (Table 25) and NA under both water regimes (Table 26), NS-1 was more likely to have higher  $N_2$  fixation under both water regimes.

## 5. Seed yield

Plants were harvested at their physiological maturity. Stressed plants took longer maturity about a week than well watered plants. Reflux of reproductive organs had occurred after re-watering at stress-plants of the all cultivars. Therefore, pods bearing without seeds and un-filled seeds were found in stressed plants of all cultivars. Therefore, only seed yield (g plant<sup>-1</sup>) of each cultivar was considered for yield and presented in (Table 27).

**Table 27** Seed yield (g plant<sup>-1</sup>) of the soybean cultivar (Cr) under well watered (Control) and re-watering after 15 days progressive soil drying (Stress) water regimes (W).

Cultivar	Control	Stress	Relative ratio
NS-1	3.34	0.77	0.23
ST-2	1.56	0.78	0.50
ST-3	3.92	0.78	0.27
Mean	2.94	0.78	0.27
LSD <sub>0.05</sub> W		0.74	
LSD <sub>0.05</sub> W x Cr		1.28	
CV%		37.8	

In a column, means followed by the same letter are not significant different at LSD<sub>0.05</sub>

Seed yield was greatly reduced about 75% by soil drying water stress compared to seed yield of control plants. ST-2 had lowest seed yield reduction since its yield potential under favorable condition was lower than other two cultivars. It is difficult to interpret for N<sub>2</sub> fixation tolerant of a cultivar based on seed yield in this experiment because it was mainly concerned on nodulation and nitrogen fixation capability under control water regime and progressive soil drying water regime. In addition, excessive soil drying and other possible nutritional disorder due to several soil washing could have been encountered which also could have greatly affected on plant growth after recovery and finally on yielding in this experiment. Further researches are needed to investigate whether N<sub>2</sub> fixation tolerant character could lead

to increased yields how much under drought stress among similar yielding genotypes without excessive soil drying and other nutrition effect.

#### 6. Relationships between nodule dry matter and other observed parameters

Evidences indicated that NDW is associated with drought tolerance in soybean (Sinclair, 1998) and it was found that NDW was significant correlation with seed yield (0.81\*\*) and shoot biomass (0.76\*\*) in the first experiment (Table 7). In this experiment, relationships between NDM and other observed physiological parameters had varied with observed times. Highest significant correlations were found at 15 DAV<sub>4</sub> (R<sub>4</sub> stage) presented in (Table 28).

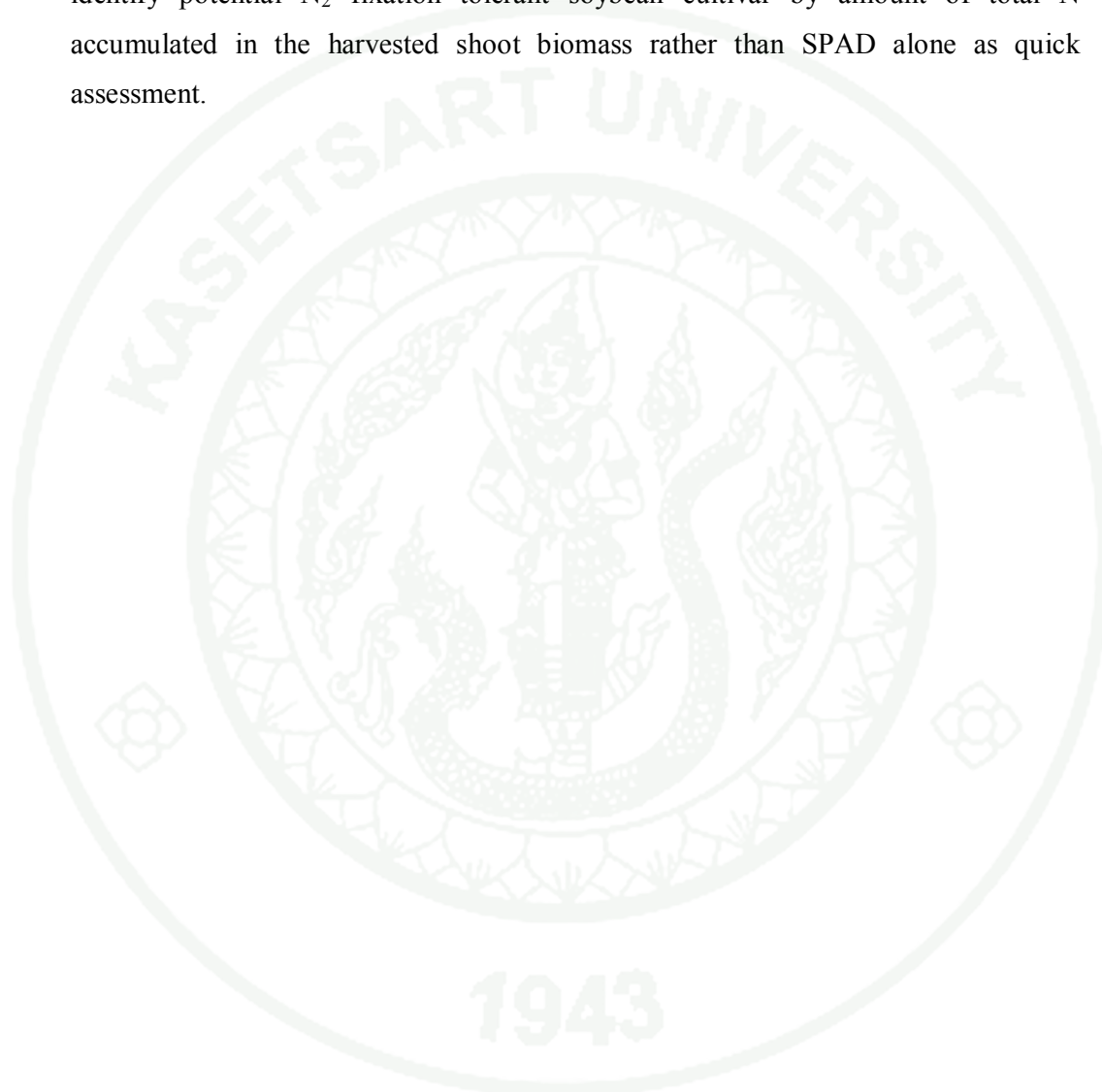
**Table 28** Simple correlation between nodule dry matter and other observed parameter at 15 DAV<sub>4</sub> (R<sub>4</sub> stage)

	NDM	NA	SDM	RDM	SPAD	AcN	Nconc <sub>7-15</sub>	SYd
NDM	1	0.91**	0.87**	0.62**	0.85**	0.91**	0.30 <sup>ns</sup>	0.65**
NA		1	0.84**	0.64**	0.84**	0.88**	0.34 <sup>ns</sup>	0.79**
SDM			1	0.64**	0.78**	0.97**	0.20 <sup>ns</sup>	0.55*
RDM				1	0.59**	0.67**	0.29 <sup>ns</sup>	0.24 <sup>ns</sup>
SPAD					1	0.82**	0.32 <sup>ns</sup>	0.73**
AcN						1	0.33 <sup>ns</sup>	0.61**
Nconc <sub>7-15</sub>							1	0.29 <sup>ns</sup>
SYd								1

NDM: nodule dry weight, NA: nitrogenase activity by acetylene reduction assay, RDM; root dry matter, SPAD; SPAD reading; AcN: accumulated total N in the shoot biomass at harvest (µg N in SDM at harvest), Nconc<sub>7-15</sub>: concentration of total N in accumulate shoot biomass between 7 and 15 DAV<sub>4</sub> harvest (µg N g<sup>-1</sup> of SDM), SdY: seed yield (g plant<sup>-1</sup>), ns : not significant, \* : significant at p≤ 0.05, \*\* and significant at p≤ 0.01, n=18

NDW had high significant correlation coefficient values with observed physiological parameters at R<sub>4</sub>, and finally with seed yield tested under different

water regimes (Table 28). SPAD readings also had good significant correlations with observed parameters. Therefore, SPAD could be used for identification of potential N<sub>2</sub> fixation soybean cultivars. However, it is more likely to be more meaningful to identify potential N<sub>2</sub> fixation tolerant soybean cultivar by amount of total N accumulated in the harvested shoot biomass rather than SPAD alone as quick assessment.



## Experiment IV

### Field grown soybean yield varied with timing of drought stress occurrence

#### 1. Some physical and chemical properties of the soil of the experimental site

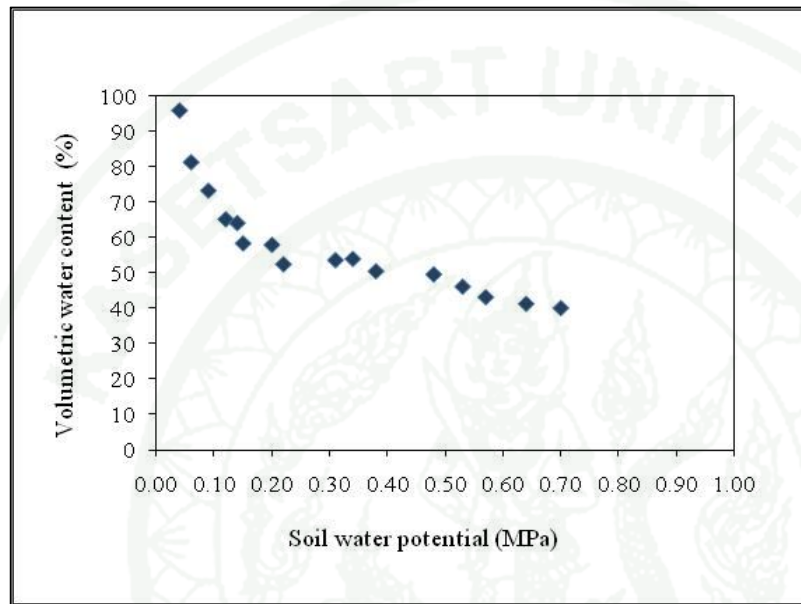
Soil samples at depth of 40 cm of the experimental site were analyzed for some physical and chemical properties. The soil was sandy loam in texture and contained 1.3% of organic matter and high content in plant available phosphate, potassium and manganese. The observed physical and chemical properties of the soil were presented in (Table 29).

**Table 29** Some physical and chemical properties of the soil of the experimental site at 40 cm depth.

<u>Physical properties</u>	
Soil texture	Sandy loam ( 57.08: 18.62: 19.34)
Volumetric soil water content at FC (%)	28.75
Volumetric soil water content at PWP (%)	6.68
<u>Chemical properties</u>	
pH(1:1)	6.42
ECe (dS/m)	1.27
OM (%)	1.35
Available phosphate (mg kg <sup>-1</sup> )	141.62
Available potassium (mg kg <sup>-1</sup> )	122
Mn (mg kg <sup>-1</sup> )	41.72

Physical properties were analyzed at Department of Soil Science, and chemical properties were analyzed at the soil science section, the Central Laboratory, Kasetsart University at Kamphaengsan,

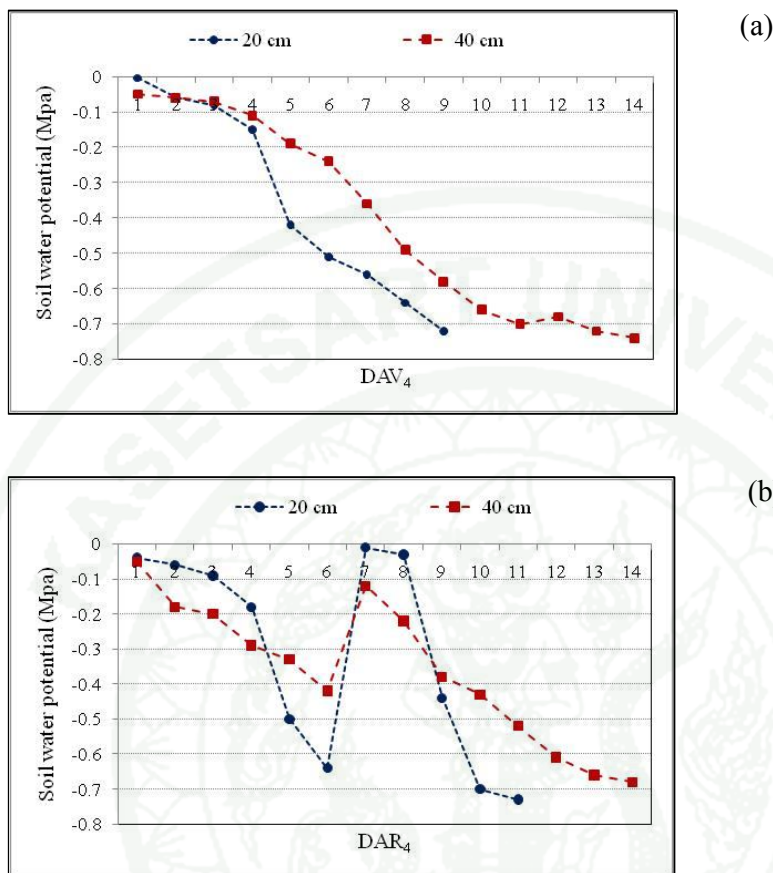
Volumetric plant available water content (%) against soil water potential was presented in (Fig.15).The previous crop was maize followed by fellow before planting.



**Figure 16** Volumetric water content against soil water potential of the soil of experimental site.

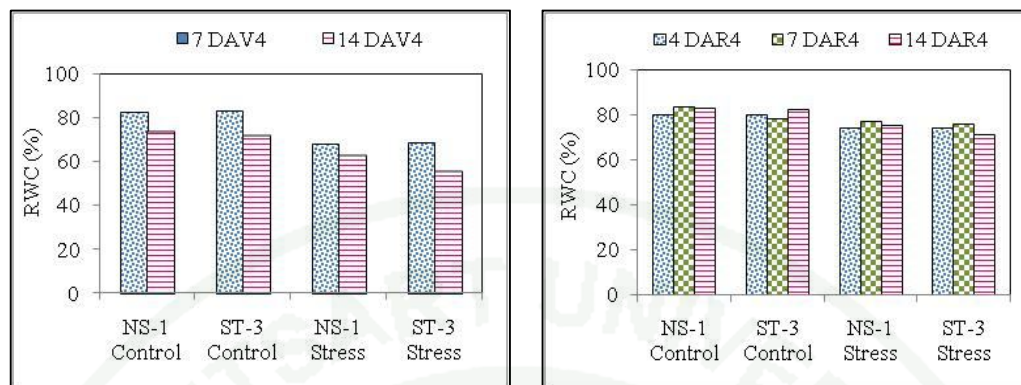
## 2. Soil and plant water status

Soil water potential decreased gradually until 4 days after withholding water (DAWW), but sharply declined up to out of detectable range (0.7 MPa) of tesiometer in first top layer (20 cm depth) at 9 DAWW in water stress period of water treatment started at V<sub>4</sub>. However, soil water sustained in deeper top soil layer (40 cm) in both withholding water stress treatments. During water stress period started at R<sub>4</sub>, soil moisture potential suddenly increased after sufficient rain had occurred at 6 DAWW and sharply declined within 2 days in the first top layer (20 cm) and gradual decreased in the second top layer (Fig 17).



**Figure 17** Changes of soil water potential during 14 days withholding water stress after V<sub>4</sub> (a) and 14 days withholding water stress after R<sub>4</sub> growth stage (b).

Relative water content of the leaf had changed with decreased in soil water potential during each withholding water stress (Fig 18). RWC of the stressed plants had significantly ( $p < 0.006$ ) since 7 DAV<sub>4</sub> and 4 DAR<sub>4</sub> of the withholding water treatments. RWC of stressed plants decreased up to 59 and 72% at 14 DAV<sub>4</sub> and 14 DAR<sub>4</sub> respectively. There was no significant difference between two cultivars and no interaction between cultivar and water treatments.

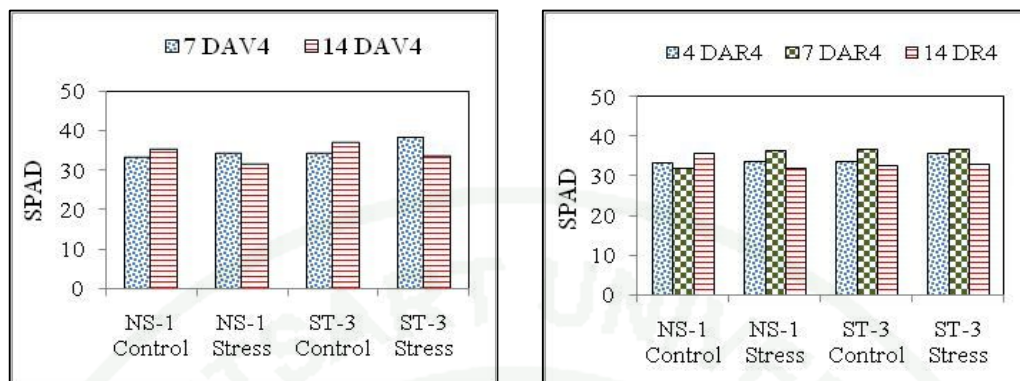


**Figure 18** RWC of the leaf of the soybean cultivars under well irrigated (Control) and withholding water (Stress) at observed times

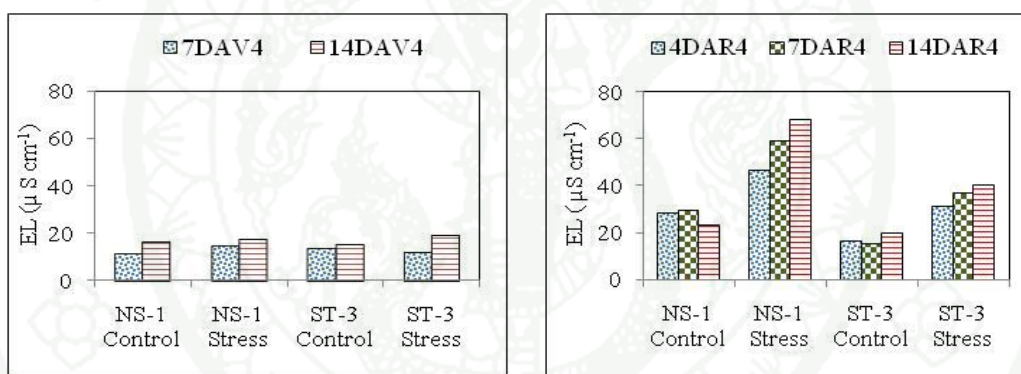
### 3. SPAD reading and electrolyte leakage of the leaf tissue

SPAD reading of the stressed plants were greater over controlled plant in early stress period but significantly less than controlled plants when stress period was prolonged (Fig 18). Trends of changes in SPAD reading were consistent with pervious experiments (Experiment II and III), generally. Decreased in SAPD indicated that reduction in total chlorophyll content which partly contributed to decreased in photosynthesis process that demonstrated in the experiment II.

EL ( $\mu S cm^{-1}$ ) of leaf tissue was increased with increased withholding water stress (Fig 19). EL of leaf tissue of stressed plants was significantly greater than controlled plants at in all observed times except 7 DAV<sub>4</sub>, indicating that cell membrane integrity was damage by imposed stress. On the other hand, increased in EL under water stress indicated that improved osmo-regulation for stomata closure to reduced transpiration water loss (Franca *et al.*, 2000; Saelim and Zwiazek, 2000; Premachandra *et al.*, 1989).



**Figure 19** SPAD reading of the leaf of the soybean cultivars under well irrigated (Control) and withholding water (Stress) at observed times.



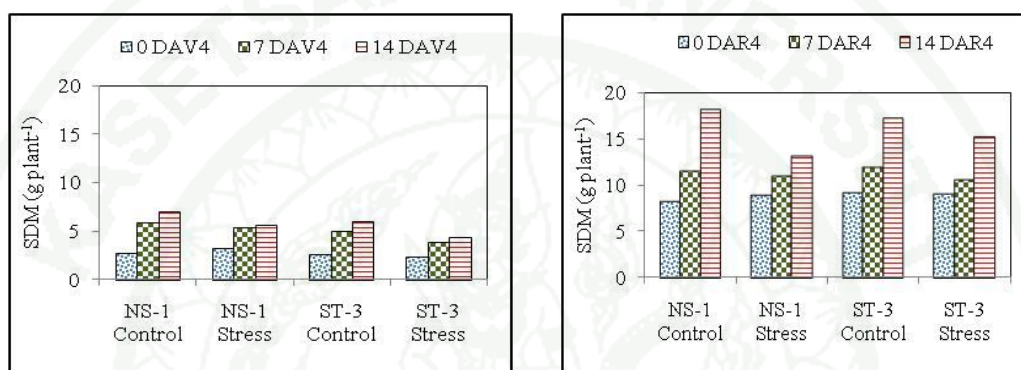
**Figure 20** EL of leaf tissue of the soybean cultivars under well irrigated (Control) and withholding water (Stress) at observed times.

Significant interaction between cultivars and water treatments were found at 4 and 7 DAR<sub>4</sub>. It has been reported that EL was changed with crop age and varied with genotypes and stress intensity (Tsarouhas *et al.*, 2000).

#### 4. Shoot biomass

Pandy *et al.* (1984) reported that increasing drought stress progressively reduced leaf area, leaf area duration (LAD), crop growth rate (CGR), and shoot dry

matter. Shoot dry matter accumulation was also decreased in withholding water stress treatments, but significant reduction was found at 14 DAR<sub>4</sub> ( $p=0.01$ ) (Fig 20). Stressed plants could attain substantial soil water through deeper root growth to maintain their growth, however, significant decreased at 14 DAR<sub>4</sub>, indicated that greater demand from seeds, therefore less allocation of photo-assimilates to shoot.



**Figure 21** Shoot dry matter (SDM) of the soybean cultivars under well irrigated (Control) and withholding water (Stress) at observed times

### 5. Yield and yield components

Seed yield of the tested soybean cultivars were significantly ( $p < 0.0001$ ) reduced in withholding water stress treatments mainly due to significant reduction in pods numbers ( $p < 0.01$ ) while other yield components were not. Tested cultivars were not significant different in responding to water treatments. However, they were genotypically difference in 100 seed weight and number of seeds per pod therefore resulted significant interaction by given water treatments (Table 30).

**Table 30** Yield and yield components of the soybean cultivars (C) under different water regimes (W).

Water regime	Cultivar	Pods plant <sup>-1</sup>	Seeds pod <sup>-1</sup>	100 seed Weight (g)	Seed yield (kg ha <sup>-1</sup> )
Control	NS-1	21.83	1.95	15.57	1789.46
	ST-3	21.23	1.63	11.36	1830.17
	Mean	21.53 <sup>a</sup>	1.79 <sup>a</sup>	13.47 <sup>a</sup>	1809.81 <sup>a</sup>
Stress <sub>V<sub>4</sub></sub>	NS-1	16.55	1.59	15.04	1347.89
	ST-3	16.67	1.68	11.44	1520.56
	Mean	16.61 <sup>b</sup>	1.63 <sup>a</sup>	13.24 <sup>a</sup>	1434.22 <sup>b</sup>
Stress <sub>R<sub>4</sub></sub>	NS-1	18.27	1.99	15.90	1352.60
	ST-3	18.66	1.54	11.22	1435.40
	Mean	18.47 <sup>b</sup>	1.77 <sup>a</sup>	13.56 <sup>a</sup>	1394.00 <sup>b</sup>
LSD <sub>0.05</sub> (W)		2.42	0.21	0.53	160.39
LSOD <sub>0.05</sub> (C)		1.98	0.17	0.43	130.96
LSD <sub>0.05</sub> (W x C)		3.43	0.30	0.74	226.82

In a column, means followed by the same letter are not significant different at LSD<sub>0.05</sub>

The most important time for soybean plants to have adequate water are during pod development and seed fill (Kranz *et al.*, 1998). In this experiment, seed yield of withholding water stress started at R<sub>4</sub> was greater than the treatment started at V<sub>4</sub> (before flowering), but was not significant different. This was affected by sufficient occurrence at 6 DAWR<sub>4</sub>. However, many reports agreed that water deficit notably diminish production when it occurs just before or during flowering (De-Bruyn *et al.*, 1995, Choi *et al.*, 1996). Drought stress occurring during early reproductive stages (R<sub>2</sub> to R<sub>4</sub>) increases the rate of pod abortion (Westgate and Peterson, 1993), leads to a less number of pods per plant (Desclaux *et al.*, 2000), and ultimately decreases seed yield (Kokubun *et al.*, 2001). Brvedan and Egli, (2003) stated that the most critical period of drought is from before flowering to seed-filling period.

## CONCLUSIONS

Among the tested soybean cultivars, cultivar SJ-4 is the most appropriate cultivar not only for cultivar improvement but also crop production in drought prone area because its physiological properties responding to drought and better yield potential in drought stress environments. ST-2 also showed potential genetic source for drought tolerant cultivar improvement in the first experiment. Among the drought tolerant indices, STI is the most appropriate yield based index for selecting drought tolerant cultivar, followed by GMP.

The specific leaf weight (SLW) showed significant correlation with photosynthetic parameters, fluorescence parameters, water use efficiencies parameters and number of seed yield; followed after shoot dry matter accumulation of a given genotype. Therefore, SLW trait is an alternative potential cost effective criterion for selecting productive genotypes for both favorable and drought stress environments.

Nitrogen fixation is sensitive to water deficit, NA of nodulated root had significantly decreased 17.73% of control since 7 DAV<sub>4</sub> (R<sub>3</sub>). Not only NA of nodulated root severely diminished 87.10% of control at 12 DAV<sub>4</sub> (R<sub>4</sub>) under progressive soil drying and did not recovered well after re-watering but also in nodule growth. Although NDM is the most fundamental parameter for selecting greater soybean cultivar for N<sub>2</sub> fixation under both water regimes, nitrogen concentration in accumulated shoot biomass under well watered could be used as an alternative indicator. SPAD (M-502) could be used as a powerful tool for selecting not only for productive genotypes but also for N<sub>2</sub> fixation drought tolerant soybean cultivars since it has significant high correlations with those potential traits; SLW, SDM and NDM. However, greater correlations were found at later reproductive growth stages of soybean.

Drought occurrence at late reproductive stage (R<sub>4</sub>) caused greater soybean seed yield reduction (23%) than occurred at V<sub>4</sub>; just before flowering (21%) even sufficient rain occurred during imposed drought period at R<sub>4</sub>.

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**APPENDICES**

**Appendix A** Some chemical properties of the garden soil used in the first experiment

Chemical properties	Value
Organic matter content	15.1%
pH	7.1
Phosphorous	137 (ppm)
Potassium	1250 (ppm)
Calcium	7600 (ppm)
Magnesium	634 (ppm)
CEC	52.1 (E mol kg <sup>-1</sup> )

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**Appendix B** Climatic conditions during the field experiment (IV), at Kamphaesaeng Saen, Nakhonpathom, (February- May, 2010).

February, 2010

Date	Air temperature (°C)		Humidity (%)		Rain (mm)	Evap (mm)	Colud (%)	Sunshine (hr)	Wind Speed Km hr <sup>-1</sup>
	Max	Mini	Max	Mini					
1	35.2	23.5	93	44	0.0	4.1	10	8.7	1.8
2	35.0	24.0	93	44	0.0	4.5	34	8.2	2.9
3	35.2	24.0	91	41	0.0	3.8	35	8.0	1.4
4	35.6	23.6	91	41	0.0	3.6	14	8.7	0.4
5	35.4	23.8	92	44	0.0	4.6	15	8.9	1.8
6	34.7	24.2	93	47	0.0	4.5	20	9.1	5.0
7	34.8	24.2	93	39	0.0	4.1	25	8.7	1.4
8	34.7	23.2	94	39	0.0	5.1	11	9.4	3.6
9	35.1	22.4	93	42	0.0	4.7	6	9.5	3.2
10	35.9	22.7	92	44	0.0	4.1	0	9.6	3.6
11	36.2	22.0	94	36	0.0	5.9	5	9.6	2.2
12	35.4	23.2	92	46	0.0	5.5	3	9.7	1.8
13	35.2	23.2	93	43	0.0	5.4	0	9.5	2.2
14	35.7	23.5	92	43	0.0	5.7	11	9.5	5.0
15	35.8	24.2	93	44	0.0	5.2	8	9.7	3.2
16	34.8	23.5	92	47	0.0	3.7	11	9.9	2.9
17	34.8	23.7	91	47	0.0	5.3	8	9.9	2.2
18	35.7	23.5	92	44	0.0	3.7	14	9.2	3.2
19	35.4	24.6	92	45	0.0	5.3	43	8.6	1.4
20	35.6	24.3	93	35	0.0	4.9	31	9.2	3.6
21	36.2	23.9	92	26	0.0	5.2	23	7.6	1.4
22	34.8	23.3	92	36	0.0	4.2	6	9.8	2.2
23	34.9	23.7	93	36	0.0	6.5	20	9.8	3.2
24	34.8	23.5	93	36	0.0	4.3	29	7.8	3.6
25	35.4	21.3	93	30	0.0	5.3	28	6.4	2.2
26	35.7	22.7	92	34	0.0	5.5	24	9.4	0.4
27	35.9	21.6	94	46	0.0	4.1	0	8.9	2.9
28	36.1	25.1	96	45	0.0	5.9	30	9.7	5.0
Total	990.0	656.4	2594	1144	0.0	134.7	464	253.0	73.7
Mean	35.4	23.4	93	41	0.0	4.8	17	9.0	2.6

## Appendix B (Continued)

March, 2010

Date	Air temperature (°C)		Humidity (%)		Rain (mm)	Evap (mm)	Colud (%)	Sunshine (hr)	Wind Speed (Km hr <sup>-1</sup> )
	Max	Mini	Max	Mini					
1	35.5	24.5	99	49	0.0	5.7	36	8.8	5.8
2	36.4	23.9	98	34	0.0	4.9	28	9.3	2.2
3	36.3	24.7	96	39	0.0	6.1	3	9.3	4.7
4	37.1	23.2	99	17	0.0	5.2	6	9.2	3.2
5	37.7	22.2	100	12	0.0	6.5	0	9.8	6.5
6	37.6	20.5	94	16	0.0	5.3	1	9.8	2.9
7	36.7	22.7	99	40	0.0	3.2	0	9.2	1.4
8	36.6	24.0	95	42	0.0	6.1	1	9.3	3.6
9	35.7	24.9	96	49	0.0	3.9	10	9.5	2.2
10	28.5	22.6	92	57	0.0	5.9	86	1.4	5.8
11	31.5	19.6	85	49	0.0	4.4	16	8.7	5.0
12	36.5	22.0	98	38	0.0	4.6	25	9.2	1.4
13	36.6	24.1	92	47	0.0	6.0	9	9.2	4.0
14	36.0	23.9	97	50	0.0	5.1	3	9.8	2.9
15	36.9	24.2	96	42	0.0	4.3	0	9.7	1.8
16	36.7	23.5	97	49	0.0	5.6	11	9.7	3.6
17	34.1	23.9	97	56	0.0	5.2	63	7.1	2.9
18	35.2	22.8	97	44	0.0	4.0	16	8.7	2.9
19	36.3	22.5	97	51	0.0	5.0	20	6.2	1.4
20	36.3	24.5	95	45	0.0	5.5	23	9.2	2.9
21	36.7	24.1	97	40	0.0	4.8	25	9.3	2.9
22	36.2	25.9	90	51	0.0	6.0	18	9.2	5.0
23	36.0	25.0	93	51	0.0	5.2	5	9.8	5.0
24	36.9	25.5	95	44	0.0	5.7	0	9.8	5.8
25	37.4	25.0	95	50	T	4.9	10	9.3	1.8
26	29.1	22.6	92	64	0.0	3.5	74	1.0	3.6
27	32.3	22.7	87	53	0.0	3.9	76	4.7	3.6
28	35.5	23.5	92	45	0.0	6.0	48	8.2	1.4
29	35.9	21.0	99	48	21.0	FULL	55	6.3	4.0
30	36.3	23.2	96	48	0.0	3.5	44	8.9	3.2
31	37.0	25.0	96	40	0.0	5.9	30	8.8	4.0
Total	1103.5	727.7	2951	1360	21.0	151.9	742	258.4	107.4
Mean	35.6	23.5	95	44	0.7	5.1	24	8.3	3.5

## Appendix B (Continued)

April, 2010

Date	Air temperature (°C)		Humidity (%)		Rain (mm)	Evap (mm)	Colud (%)	Sunshine (hr)	Wind Speed Km hr <sup>-1</sup>
	Max	Max	Maxi	Mini					
1	37.8	25.6	96	43	0.0	6.0	9	9.2	4.7
2	36.8	25.6	96	47	0.0	5.0	9	10.1	4.0
3	37.2	26.1	97	46	0.0	5.0	10	10.0	2.2
4	37.2	26.4	97	46	0.0	6.0	19	9.8	2.2
5	37.9	25.8	94	47	0.0	7.2	8	10.6	2.2
6	37.5	25.7	96	49	0.0	6.2	18	10.0	2.2
7	37.5	25.6	97	50	0.0	5.8	26	10.2	2.2
8	37.5	24.8	97	49	0.0	4.1	36	9.5	2.2
9	38.3	26.0	96	37	0.0	5.0	33	9.5	3.6
10	38.6	25.6	96	32	0.0	6.0	24	9.2	5.8
11	39.3	25.7	98	29	0.0	4.7	9	10.3	4.7
12	39.3	26.0	96	24	0.0	6.4	0	10.3	1.8
13	39.0	24.3	96	25	0.0	6.2	5	9.2	2.2
14	37.8	25.2	96	47	0.0	5.6	4	10.6	5.0
15	38.1	26.5	97	45	0.0	5.4	11	10.6	4.0
16	37.1	26.9	95	52	5.2	7.5	43	8.2	5.8
17	32.4	25.1	98	66	0.3	3.1	89	0.5	3.6
18	36.1	25.2	96	52	0.0	6.6	31	9.9	4.0
19	37.5	24.8	95	44	0.0	6.4	33	10.4	4.0
20	38.4	24.4	89	29	0.0	4.2	3	10.8	5.0
21	39.0	25.2	96	31	0.0	5.2	0	10.6	4.7
22	39.1	26.4	93	36	0.0	6.5	4	10.8	6.8
23	38.3	26.6	97	46	0.0	4.1	15	10.0	5.4
24	31.9	23.5	99	72	14.1	3.8	78	1.6	1.8
25	36.2	25.5	97	49	0.0	6.2	21	10.8	4.0
26	37.1	25.7	97	43	0.0	6.1	41	10.7	1.1
27	36.4	24.6	97	52	0.0	5.1	70	8.0	4.0
28	36.7	25.0	98	49	0.0	5.7	69	6.0	3.2
29	37.3	25.7	97	48	0.0	5.9	38	10.5	3.6
30	36.8	24.8	96	48	0.0	6.1	50	10.6	2.9
Total	1120.1	764.3	2885	1333	19.6	167.1	806	278.5	108.9
Mean	37.3	25.5	96	44	0.7	5.6	27	9.3	3.6

## Appendix B (Continued)

May, 2010

Date	Air temperature (°C)		Humidity (%)		Rain (mm)	Evap (mm)	Colud (%)	Sunshine (hr)	Wind Speed Km hr <sup>-1</sup>
	Max	Max	Maxi	Mini					
1	37.1	25.0	95	51	4.7	5.4	54	6.7	2.9
2	36.4	25.1	98	52	0.0	5.1	50	9.3	1.1
3	36.7	26.3	94	52	0.0	6.0	48	10.2	5.4
4	38.2	26.5	95	45	1.3	5.4	74	7.8	5.4
5	37.6	24.7	96	49	0.0	6.1	56	10.0	5.0
6	38.3	26.7	95	49	0.0	6.0	60	9.0	2.2
7	38.6	26.7	95	50	0.0	6.1	46	10.5	6.8
8	39.5	26.6	96	40	0.0	7.2	26	11.2	5.8
9	40.0	26.8	95	44	0.0	7.7	31	11.0	5.4
10	40.2	26.9	96	43	0.0	8.7	24	11.0	5.0
11	37.4	28.1	98	57	T	6.7	49	8.3	5.4
12	33.8	24.5	100	68	0.0	2.5	85	0.5	3.6
13	38.0	24.7	99	48	0.0	7.4	20	10.9	2.9
14	38.6	25.9	94	48	0.0	6.6	26	10.5	3.6
15	38.2	26.4	96	49	0.0	5.7	51	8.7	3.6
16	35.6	26.6	96	60	T	4.9	68	8.2	3.6
16	35.6	26.6	96	60	T	4.9	68	8.2	3.6
17	33.0	26.6	98	70	2.0	2.8	75	2.1	1.1
18	37.2	24.5	98	50	0.0	5.5	70	9.7	2.2
19	36.7	25.9	95	55	0.0	6.6	66	9.7	4.0
20	36.9	24.8	96	52	0.0	5.7	58	9.1	4.7
21	38.5	26.1	95	45	1.0	7.8	58	9.6	2.9
22	37.7	26.2	93	48	0.0	6.6	71	7.3	4.7
23	37.8	26.6	95	52	1.2	4.6	81	7.8	8.3
24	38.3	25.2	98	47	3.3	7.0	85	8.2	7.2
25	36.9	24.0	98	50	22.4	6.2	80	8.5	4.7
26	32.7	23.2	96	60	5.5	3.0	98	1.7	4.7
27	36.2	24.0	98	53	20.6	FULL	84	7.2	3.2
28	35.5	24.0	97	57	1.0	4.2	83	6.1	3.2
29	35.7	24.9	96	54	0.0	4.9	75	7.0	5.8
30	36.4	25.2	95	54	1.2	5.9	70	9.2	5.4
31	36.9	24.5	96	51	T	6.0	58	8.8	5.0
Total	1150.6	793.2	2982	1603	64.2	174.3	1880	255.8	134.8
Mean	37.1	25.6	96	52	2.1	5.8	60.9	8.3	4.4

Source: Nakhonpathom, Meteorological Station

**Appendix C** Balance ANOVA for Variate (Mean square) of the observed parameters

**Experiment I**

Balance ANOVA for Variate (Mean square) of tissue moisture percentage of the soybean cultivars (Cultivar) under different water regimes (Water) at observed time .

Source of variation	DF	0 DAV <sub>3</sub>	5 DAV <sub>3</sub>	10 DAV <sub>3</sub>	15 DAV <sub>3</sub>
Replication	2	3.48	2.07	10.60	8.96
Water	1	6.33	3.30	248.26	1793.57**
Cultivar	6	440.21**	14.94**	44.56**	4115**
Water x Cultivar	6	3.73	774541	11.86	24.54**
Residual	26	3.94	1.30	3.88	4.71
Total	41	68.52	3.31	17.29	56.78

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

Balance ANOVA for Variate (Mean square) of soil moisture percentage of the soybean cultivars (Cultivar) under drought water regime at observed time.

Source of variation	DF	5 DAV <sub>3</sub>	10 DAV <sub>3</sub>	15 DAV <sub>3</sub>
Replication	2	6.49	26.54	2.86
Cultivar	6	27.10	101.48**	3.83
Residual	12	13.27	17.90	2.19
Total	20	16.27	43.84	2.75

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

**Appendix C (Continued)**

Balance ANOVA for Variate (Mean square) of osmotic potential of the leaf of the soybean cultivars (Cultivar) under different water regimes (Water) at observed time.

Source of variation	DF	2 DAV <sub>3</sub>	5 DAV <sub>3</sub>	7DAV <sub>3</sub>	9 DAV <sub>3</sub>
Replication	2	0.30E-01	0.97E-02	0.89E-02	0.23E-01
Water	1	0.19E-02	0.79E-02	0.68E-02	0.28E-01
Cultivar	6	0.11E-01	0.66E-01**	0.12E-01	0.28E-01
Water x Cultivar	6	0.13E-01	0.18E-02	0.20E-02	0.18E-01
Residual	26	0.11E-01	0.56E-02	0.74E-02	0.94E-02
Total	41	0.13E-01	0.13E-01	0.75E-02	0.15E-01

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

Balance ANOVA for Variate (Mean square) of osmotic potential of the leaf of the soybean cultivars (Cultivar) under different water regimes (Water) at observed time

Source of variation	DF	11 DAV <sub>3</sub>	14 DAV <sub>3</sub>	17 DAV <sub>3</sub>	15 DAV <sub>3</sub>
Replication	2	0.24E-01	0.15E-01	0.36E-01	0.99E-01
Water	1	0.12**	0.39**	0.11*	0.76**
Cultivar	6	0.22E-02	0.39E-01*	0.26E-01	0.14
Water x Cultivar	6	0.62E-01	0.24E-01	0.25E-02	0.09
Residual	26	0.12E-01	0.72E-02	0.12E-01	0.05
Total	41	0.42	0.36E-01	0.21E-01	0.11

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

**Appendix C (Continued)**

Balance ANOVA for Variate (Mean square) of electrolyte leakage percentage (EL%) of the leaf tissue of the soybean cultivars (Cultivar) under different water regimes (Water) at observed time.

Source of variation	DF	0 DAV <sub>3</sub>	5 DAV <sub>3</sub>	10 DAV <sub>3</sub>	15 DAV <sub>3</sub>
Replication	2	0.31E-01	0.74E-02	0.44E-01	0.38E-02
Water	1	0.69E-01	0.61E-01**	1.23**	22.73**
Cultivar	6	0.37**	0.17	0.11**	2.32**
Water x Cultivar	6	0.37E-01	0.23E-01	0.21**	2.10**
Residual	26	0.27E-01	0.18E-01	0.17E-01	0.36E-01
Total	41	0.80E-01	0.12E-01	0.89E-01	1.22

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

Balance ANOVA for Variate (Mean square) of shoot dry matter (mg plant<sup>-1</sup>) of the soybean cultivars (Cultivar) under different water regimes (Water) at observed time.

Source of variation	DF	0 DAV <sub>3</sub>	5 DAV <sub>3</sub>	10 DAV <sub>3</sub>	15 DAV <sub>3</sub>
Replication	2	19904.60	30337.20	162120.0	131265
Water	1	227.11	42731.00	788024**	0.13E+08**
Cultivar	6	226121.00**	718698.0**	0.12E+07**	0.11E+07**
Water x Cultivar	6	3596.24	20811.2	87792.0	505446*
Residual	26	11401.40	45649.3	65443.5	149330
Total	41	41823.70	139691.0	255338.0	64452

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

**Appendix C (Continued)**

Balance ANOVA for Variate (Mean square) of root dry matter (g plant<sup>-1</sup>) of the soybean cultivars (Cultivar) under different water regimes (Water) at observed time.

Source of variation	DF	0 DAV <sub>3</sub>	5 DAV <sub>3</sub>	10 DAV <sub>3</sub>	15 DAV <sub>3</sub>
Replication	2	119.18	725.57	2375.06	53.53
Water	1	46.80	2757.78	104534**	11.52
Cultivar	6	2380.28**	21231.0**	36925.2**	30879.3**
Water x Cultivar	6	170.85	2003.35	5211.16	2962.65
Residual	26	176.64	4239.88	5225.99	5269.85
Total	41	492.31	6191.51	12145.8	8297.22

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

Balance ANOVA for Variate (Mean square) of nodule dry matter (mg plant<sup>-1</sup>) of the soybean cultivars (Cultivar) under different water regimes (Water) at observed time.

Source of variation	DF	0 DAV <sub>3</sub>	5 DAV <sub>3</sub>	10 DAV <sub>3</sub>	15 DAV <sub>3</sub>
Replication	2	316.22	389.77	602.62	873.27
Water	1	1.79	214.88	1173.43	85922.2**
Cultivar	6	1746.87**	1559.78**	2355.52*	1555.32
Water x Cultivar	6	101.53	167.34	386.50	2209.35
Residual	26	152.59	302.14	737.74	1212.93
Total	41	382.73	468.60	927.13	3458.36

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

**Appendix C (Continued)**

Balance ANOVA for Variate (Mean square) of seed yield and yield components of the soybean cultivars (Cultivar) under different water regimes (Water) at observed time.

Source of variation	DF	Number of pods plant <sup>-1</sup>	Number of seeds pod <sup>-1</sup>	100 weight (g)	seed yield (g plant <sup>-1</sup> )
Replication	2	0.67	0.47E-01	3.95	45.0107
Water	1	219.75**	0.11	4.71	13E+06**
Cultivar	6	10.93**	0.19**	16.18**	76E+04**
Water x Cultivar	6	9.31**	0.46E-01	2.32	10E+05**
Residual	26	1.81	0.31E-01	1.44	11E+04
Total	41	9.50	0.56E-01	3.93	65E+04

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

Balance ANOVA for Variate (Mean square) of shoot, root and nodule dry matter accumulation rate of the soybean cultivars (Cultivar) between 0-15 DAV<sub>3</sub> under different water regimes (Water) at observed time.

Source of variation	DF	Shoot dry matter accumulation rate (g plant <sup>-1</sup> day <sup>-1</sup> )	Root dry matter accumulation rate (g plant <sup>-1</sup> day <sup>-1</sup> )	Nodule dry matter accumulation rate (mg plant <sup>-1</sup> day <sup>-1</sup> )
Replication	2	0.45E-03	0.21E-05	0.35E-01
Water	1	0.59E-01**	0.15E-05	9.35**
Cultivar	6	0.65E-02**	0.59E-04**	0.62*
Water x Cultivar	6	0.15E-02	0.32E-05	0.19
Residual	26	0.12E-02	0.37E-05	0.20
Total	41	0.34E-02	0.12E-04	0.47

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

**Appendix C (Continued)**

Balance ANOVA for Variate (Mean square) of seed yield under controlled (YP) and seed yield under drought stress (YS) of the soybean cultivars (Cultivar).

Source of variation	DF	YP (g plant <sup>-1</sup> )	YS(g plant <sup>-1</sup> )
Replication	2	1.30	1.94
Cultivar	6	13.08**	4.99**
Residual	12	0.89	0.97
Total	20	4.59	2.27

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

Balance ANOVA for Variate (Mean square) of drought tolerant indices of the soybean cultivars (Cultivar).

Source of variation	DF	SSI	TOL	MP	GMP	STI
Replication	2	0.38	5.58	0.23	0.33	0.95E-02
Cultivar	6	0.85**	20.80**	3.83**	3.11**	0.76E-01**
Residual	12	0.13	2.26	0.36	0.42	0.10E-01
Total	20	0.37	8.15	1.39	1.22	0.30E-01

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

**Appendix C (Continued)****Experiment II**

Balance ANOVA for Variate (Mean square) of relative water content (%) in the leaf of the soybean cultivars (Cultivar) under different water regimes (Water) at observed time.

Source of variation	DF	5 DAV <sub>3</sub>	10 DAV <sub>3</sub>	15 DAV <sub>3</sub>	20DAV <sub>3</sub>
Replication	2	0.44E-03	0.32R-04	0.52E-02	0.27E-02
Water	1	0.17E-03	0.76E-04	0.38E-03	0.17**
Cultivar	2	0.16E-02	0.12E-02	0.24E-03	0.13E-02
Water x Cultivar	2	0.19E-02	0.22E-03	0.31E-03	0.43E-02*
Residual	10	0.75E-03	0.81E-03	0.30E-02	0.97E-03
Total	17	0.92E-03	0.65E-03	0.25E-02	0.12E-01

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

Balance ANOVA for Variate (Mean square) of soil moisture percentage in the pot of the soybean cultivars (Cultivar) under progressive soil dry water regime at observed time.

Source of variation	DF	5 DAV <sub>3</sub>	10 DAV <sub>3</sub>	15 DAV <sub>3</sub>	20 DAV <sub>3</sub>
Replication	2	10.24	11.48	7.32	1.91
Cultivar	2	26.85	1.02	14.47	5.70
Residual	4	10.81	10.53	5.83	1.73
Total	8	14.68	8.40	8.36	2.77

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

**Appendix C (Continued)**

Balance ANOVA for Variate (Mean square) of relative water content(%) in the leaf of the soybean cultivars (Cultivar) under different water regimes (Water) at observed time.

Source of variation	DF	5 DAV <sub>3</sub>	10 DAV <sub>3</sub>	15 DAV <sub>3</sub>	20 DAV <sub>3</sub>
Replication	2	0.48E-03	0.32E-04	0.52E-02	0.27E-02
Water	1	0.17E-03	0.76E-04	0.38E-03	0.17
Cultivar	2	0.16E-02	0.12E-02	0.24E-03	0.13E-02
Water x Cultivar	2	0.19E-02	0.22E-03	0.31E-03	0.44E-02
Residual	10	0.75E-03	0.81E-03	0.30E-02	0.97E-03
Total	17	0.92E-03	0.65E-03	0.25E-02	0.12E-01

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

Balance ANOVA for Variate (Mean square) of shoot dry matter (g plant<sup>-1</sup>) of the soybean cultivars (Cultivar) under different water regimes (Water) at observed time.

Source of variation	DF	0 DAV <sub>3</sub>	5 DAV <sub>3</sub>	10 DAV <sub>3</sub>	15 DAV <sub>3</sub>	20 DAV <sub>3</sub>
Replication	2	0.26E-02	0.43E-01	0.19E-01	0.86E-01	0.25E-01
Water	1	0.13E-02	0.31E-01	0.16E-04	2.55**	10.49**
Cultivar	2	0.37E01**	0.20**	0.63**	0.67E-01	0.25**
Water x Cultivar	2	0.62E-02	0.17E-01	0.63E-02	0.42E-01	0.32**
Residual	10	0.35E-01	0.12E-01	0.25E-01	0.27E-01	0.27E-01
Total	17	0.75E-02	0.40E-01	0.92E-01	0.19	0.70

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$

**Appendix C (Continued)**

Balance ANOVA for Variate (Mean square) of specific leaf area (cm<sup>2</sup>) of the soybean cultivars (Cultivar) under different water regimes (Water) at observed time.

Source of variation	DF	0 DAV <sub>3</sub>	5 DAV <sub>3</sub>	10 DAV <sub>3</sub>	15 DAV <sub>3</sub>	20 DAV <sub>3</sub>
Replication	2	55.68	57.78	194.64	174.75	411.60
Water	1	52.26	4.05	86.07	119.35	37.56
Cultivar	2	36.31	1179.05	733.68**	819.07**	812.89*
Water x Cultivar	2	9.50	15.28	145.04	50.33	86.21
Residual	10	37.06	179.01	77.41	107.56	122.69
Total	17	36.82	252.84	176.41	193.13	228.58

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

Balance ANOVA for Variate (Mean square) of specific leaf weight (mg cm<sup>-2</sup>) of the soybean cultivars (Cultivar) under different water regimes (Water) at observed time.

Source of variation	DF	0 DAV <sub>3</sub>	5 DAV <sub>3</sub>	10 DAV <sub>3</sub>	15 DAV <sub>3</sub>	20 DAV <sub>3</sub>
Replication	2	0.24E-01	0.24E-02	0.17*	0.01	0.09
Water	1	0.27E-01	0.26E-03	0.88**	0.49*	4.58**
Cultivar	2	1.93**	0.22**	2.33**	1.30**	1.11**
Water x Cultivar	2	0.72E-01	0.21E-01	0.48**	0.12	0.02
Residual	10	0.662	0.10E-01	0.03	0.09	0.06
Total	17	4.56	0.34E-01	0.41	0.25	0.45

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$

**Appendix C (Continued)**

Balance ANOVA for Variate (Mean square) of SPAD reading of the second most fully expanded leaf of the soybean cultivars (Cultivar) under different water regimes (Water) at observed time.

Source of variation	DF	0 DAV <sub>3</sub>	5 DAV <sub>3</sub>	10 DAV <sub>3</sub>	15 DAV <sub>3</sub>	20 DAV <sub>3</sub>
Replication	2	7.39	3.24	4.09	7.48	10.20
Water	1	2.00	0.41	0.01	8.54	282.43**
Cultivar	2	20.82	1.90	9.39	28.17*	20.16*
Water x Cultivar	2	17.33	14.85**	1.08	0.27	17.39
Residual	10	7.58	1.44	3.75	5.27	4.51
Total	17	9.35	3.22	3.92	7.83	48.89

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

Balance ANOVA for Variate (Mean square) of Chl<sub>a</sub> content ( $\mu\text{g cm}^{-2}$ ) in the second most fully expanded leaf of the soybean cultivars (Cultivar) under different water regimes (Water) at observed time.

Source of variation	DF	0 DAV <sub>3</sub>	5 DAV <sub>3</sub>	10 DAV <sub>3</sub>	15 DAV <sub>3</sub>	20 DAV <sub>3</sub>
Replication	2	9.53	19.27	8.01	13.11	2.50
Water	1	4.66	1.95	29.75	49.29	646.03**
Cultivar	2	43.12*	19.36	164.9**	354.60**	435.35**
Water x Cultivar	2	25.14	9.36	12.95	6.79	214.76**
Residual	10	10.56	12.05	12.20	14.33	24.10
Total	17	15.64	12.88	30.79	55.39	128.96

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

**Appendix C (Continued)**

Balance ANOVA for Variate (Mean square) of  $\text{Chl}_b$  content ( $\mu\text{g cm}^{-2}$ ) in the second most fully expanded leaf of the soybean cultivars (Cultivar) under different water regimes (Water) at observed time.

Source of variation	DF	0 DAV <sub>3</sub>	5 DAV <sub>3</sub>	10 DAV <sub>3</sub>	15 DAV <sub>3</sub>	20 DAV <sub>3</sub>
Replication	2	0.91	0.86	0.55	1.73	1.55
Water	1	0.24	0.42	2.19	1.18	27.40*
Cultivar	2	3.02	7.22**	16.14**	24.72**	44.87**
Water x Cultivar	2	1.68	4.22*	1.01	0.05	19.38*
Residual	10	0.83	0.67	1.11	1.57	3.99
Total	17	1.16	1.87	2.87	4.11	11.70

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

Balance ANOVA for Variate (Mean square) of  $\text{Chl}_t$  content ( $\mu\text{g cm}^{-2}$ ) in the second most fully expanded leaf of the soybean cultivars (Cultivar) under different water regimes (Water) at observed time.

Source of variation	DF	0 DAV <sub>3</sub>	5 DAV <sub>3</sub>	10 DAV <sub>3</sub>	15 DAV <sub>3</sub>	20 DAV <sub>3</sub>
Replication	2	16.32	27.69	12.22	23.55	7.60
Water	1	7.03	4.18	48.07	65.75	939.55**
Cultivar	2	68.90	49.28	284.15**	566.54**	752.57**
Water x Cultivar	2	39.81	26.08	21.14	6.38	362.36
Residual	10	17.18	14.98	20.31	25.14	46.27
Total	17	25.23	21.18	52.13	88.83	214.55

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

**Appendix C (Continued)**

Balance ANOVA for Variate (Mean square) of chlorophyll a/b in the second most fully expanded leaf of the soybean cultivars (Cultivar) under different water regimes (Water) at observed time.

Source of variation	DF	0 DAV <sub>3</sub>	5 DAV <sub>3</sub>	10 DAV <sub>3</sub>	15 DAV <sub>3</sub>	20 DAV <sub>3</sub>
Replication	2	0.23E-01	0.24	0.14E-01	0.27E-01	0.04
Water	1	0.74E-03	0.20	0.77E-06	0.83E-01*	0.61**
Cultivar	2	0.12E-02	0.91	0.20E-01	0.10E-01	0.23**
Water x Cultivar	2	0.62E-02	0.68	0.24E-02	0.79E-01**	0.04
Residual	10	0.64E-01	0.38	0.10E-01	0.10E-01	0.06
Total	17	0.17E-01	0.45	0.10E-01	0.24E-01	0.11

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

Balance ANOVA for Variate (Mean square) of maximum photosynthesis ( $\mu\text{mol of CO}_2 \text{ m}^{-2} \text{ S}^{-1}$ ) in the second most fully expanded leaf of the soybean cultivars (Cultivar) under different water regimes (Water) at observed time.

Source of variation	DF	5 DAV <sub>3</sub>	10 DAV <sub>3</sub>	15 DAV <sub>3</sub>	20 DAV <sub>3</sub>
Replication	2	23.34	1.61	60.77	44.34
Water	1	10.42	23.81*	455.82**	1006.90**
Cultivar	2	31.30**	16.01*	87.15*	42.55*
Water x Cultivar	2	4.89	0.73	44.18	39.54
Residual	10	5.51	3.89	13.12	10.59
Total	17	10.85	5.85	50.78	80.33

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

**Appendix C (Continued)**

Balance ANOVA for Variate (Mean square) of stomata conductance ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) in the second most fully expanded leaf of the soybean cultivars (Cultivar) under different water regimes (Water) at observed time.

Source of variation	DF	5 DAV <sub>3</sub>	10 DAV <sub>3</sub>	15 DAV <sub>3</sub>	20 DAV <sub>3</sub>
Replication	2	0.06	0.42E-01	0.11E-01	0.65E-02
Water	1	0.02	0.12**	0.48**	0.21**
Cultivar	2	0.08*	0.55E-01*	0.59E-01*	0.35E-01**
Water x Cultivar	2	0.01	0.22E-02	0.49E-01	0.62E-01*
Residual	10	0.07	0.92E-02	0.11E-01	0.43E-02
Total	17	0.24	0.25E-02	0.49E-01	0.23E-01

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

Balance ANOVA for Variate (Mean square) of inter-cellular CO<sub>2</sub> concentration ( $\mu\text{mol mol}^{-1}$ ) in the second most fully expanded leaf of the soybean cultivars (Cultivar) under different water regimes (Water) at observed time.

Source of variation	DF	5 DAV <sub>3</sub>	10 DAV <sub>3</sub>	15 DAV <sub>3</sub>	20 DAV <sub>3</sub>
Replication	2	793.5	472.7	1197.4	53766.2
Water	1	868.1	2713.4**	43218.0**	30718.9
Cultivar	2	668.2	742.1	2397.7	23927.2
Water x Cultivar	2	137.7	137.1	3253.5	14014.4
Residual	10	203.9	264.5	1672.9	19537.2
Total	17	359.2	474.3	4331.9	24088.6

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

**Appendix C (Continued)**

Balance ANOVA for Variate (Mean square) of transpiration ( $E$ ; mol of  $H_2O$   $m^{-2} s^{-1}$ ) in the second most fully expanded leaf of the soybean cultivars (Cultivar) under different water regimes (Water) at observed time different water regimes (Water) at observed time.

Source of variation	DF	5 DAV <sub>3</sub>	10 DAV <sub>3</sub>	15 DAV <sub>3</sub>	20 DAV <sub>3</sub>
Replication	2	3.49	6.36	2.40	1.27
Water	1	0.97	7.12*	61.01**	52.32**
Cultivar	2	2.73*	4.16*	5.17	6.34*
Water x Cultivar	2	0.52	0.29	6.10	5.03*
Residual	10	0.43	0.94	1.81	0.88
Total	17	1.10	2.24	6.26	5.08

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

Balance ANOVA for Variate (Mean square) of  $\Phi$ PSII in photochemical process in the second most fully expanded leaf of the soybean cultivars (Cultivar) under different water regimes (Water) at observed time.

Source of variation	DF	5 DAV <sub>3</sub>	10 DAV <sub>3</sub>	15 DAV <sub>3</sub>	20 DAV <sub>3</sub>
Replication	2	0.13E-03	0.40E-03	0.14E-03	0.91E-03
Water	1	0.26E-02	0.73E-03	0.18E-01**	0.89E-01**
Cultivar	2	0.27E-03	0.85E-03	0.12E-01**	0.41E-02
Water x Cultivar	2	0.85E-03	0.19E-03	0.34E-02*	0.34E-02
Residual	10	0.79E-03	0.62E-03	0.71E-03	0.16E-02
Total	17	0.90E-03	0.57E-03	0.34E-02	0.72E-02

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

**Appendix C (Continued)**

Balance ANOVA for Variate (Mean square) of PSII trapping efficiency ( $F_v/F_m$ ) in photochemical process in the second most fully expanded leaf of the soybean cultivars (Cultivar) under different water regimes (Water) at observed time.

Source of variation	DF	5 DAV <sub>3</sub>	10 DAV <sub>3</sub>	15 DAV <sub>3</sub>	20 DAV <sub>3</sub>
Replication	2	0.20E-04	0.45E-03	0.49E-03	0.21E-03
Water	1	0.85E-03	0.23E-03*	0.16E-01*	0.11E-01*
Cultivar	2	0.85E-03	0.18E-02	0.25E-02	0.39E-02
Water x Cultivar	2	0.10E-03	0.64E-04	0.50E-02	0.19E-02
Residual	10	0.59E-03	0.27E-03	0.17E-02	0.16E-02
Total	17	0.51E-02	0.36E-03	0.29E-02	0.23E-02

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

Balance ANOVA for Variate (Mean square) of photochemical quenching ( $q_p$ ) in photochemical process in the second most fully expanded leaf of the soybean cultivars (Cultivar) under different water regimes (Water) at observed time.

Source of variation	DF	5 DAV <sub>3</sub>	10 DAV <sub>3</sub>	15 DAV <sub>3</sub>	20 DAV <sub>3</sub>
Replication	2	0.28E-03	0.12E-02	0.73E-03	0.43E-02
Water	1	0.46E-02	0.16E-02	0.17E-01*	0.29**
Cultivar	2	0.18E-04	0.47E-04	0.29E-01**	0.63E-02
Water x Cultivar	2	0.35E-02	0.11E-02	0.52E-02	0.69E-02
Residual	10	0.27E-02	0.23E-02	0.18E-02	0.30E-02
Total	17	0.26E-02	0.18E-02	0.61E-02	0.21

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

**Appendix C (Continued)**

Balance ANOVA for Variate (Mean square) of ETR in photochemical process in the second most fully expanded leaf of the soybean cultivars (Cultivar) under different water regimes (Water) at observed time different water regimes (Water) at observed time.

Source of variation	DF	5 DAV <sub>3</sub>	10 DAV <sub>3</sub>	15 DAV <sub>3</sub>	20 DAV <sub>3</sub>
Replication	2	31.41	110.37	33.89	225.79
Water	1	658.78	187.28	4728.16**	23256.0**
Cultivar	2	69.79	209.47	3187.11**	1070.50
Water x Cultivar	2	219.76	48.93	888.79*	904.41
Residual	10	205.64	159.83	182.90	417.10
Total	17	232.12	147.13	869.22	1872.26

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

Balance ANOVA for Variate (Mean square) of intrinsic water use efficiency in the second most fully expanded leaf of the soybean cultivars (Cultivar) under different water regimes (Water) at observed time different water regimes (Water) at observed time.

Source of variation	DF	5 DAV <sub>3</sub>	10 DAV <sub>3</sub>	15 DAV <sub>3</sub>	20 DAV <sub>3</sub>
Replication	2	148.715	66.25	740.79	18635.9
Water	1	301.24	1302.83**	19304.0**	10901.8
Cultivar	2	279.67	416.19	1144.17	7379.17
Water x Cultivar	2	54.76	132.82	1477.37	3861.95
Residual	10	155.62	103.80	680.68	8491.0
Total	17	166.10	210.08	1931.49	9129.76

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

**Appendix C (Continued)**

Balance ANOVA for Variate (Mean square) of plant water use efficiency of the soybean cultivars (Cultivar) under different water regimes (Water) under different water regimes (Water) within observed time –interval.

Source of variation	DF	0-5 DAV <sub>3</sub>	6-10 DAV <sub>3</sub>	11-15 DAV <sub>3</sub>	16-20 DAV <sub>3</sub>	0-20 DAV <sub>3</sub>
Replication	2	0.77E-01	0.22	0.29	1.97	0.06
Water	1	0.20	0.06	1.97*	11.52*	0.06
Cultivar	2	0.17	0.99*	1.32	0.67	0.01
Water x Cultivar	2	0.25E-01	0.38	0.22	6.88	0.05
Residual	10	0.84E-01	0.19	0.34	1.74	0.02
Total	17	0.93E-01	0.30	0.53	2.825	0.28

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

Balance ANOVA for Variate (Mean square) seed yield and yield components of the soybean cultivars (Cultivar) under different water regimes (Water) at observed time.

Source of variation	DF	Number of pods plant <sup>-1</sup>	Number of seeds pod <sup>-1</sup>	100 seed weight (g)	Seed yield (g plant <sup>-1</sup> )
Replication	2	0.61E-02	0.01	0.22	0.02
Water	1	184.74**	5.21**	2.77*	10.52**
Cultivar	6	39.74**	0.15	14.92**	2.88**
Water x Cultivar	6	17.79**	0.19	0.81	0.55**
Residual	26	0.93	0.08	0.45	0.05
Total	41	18.19	0.39	2.30	1.05

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

**Appendix C (Continued)****Experiment III**

Balance ANOVA for Variate (Mean square) of relative water content (%) in the leaf of the soybean cultivars (Cultivar) under different water regimes (Water) at observed time.

Source of variation	DF	7DAV <sub>4</sub>	12 DAV <sub>4</sub>	15 DAV <sub>4</sub>
Replication	2	1.64	9.54	58.17
Water	1	3470.18**	4427.12**	5585.74**
Cultivar	2	22.14	31.79	49.54
Water x Cultivar	2	24.79	59.63	47.99
Residual	10	8.56	27.95	24.81
Total	17	214.88	288.74	361.49

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

Balance ANOVA for Variate (Mean square) of soil moisture percentage in the pot of the soybean cultivars (Cultivar) under progressive soil dry water regime at observed time.

Source of variation	DF	7DAV <sub>4</sub>	12 DAV <sub>4</sub>	15 DAV <sub>4</sub>
Replication	2	18.44	0.92	1.35
Cultivar	2	32.59	0.29	0.48
Residual	4	4.68	0.57	0.27
Total	8	15.10	0.59	0.59

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

**Appendix C (Continued)**

Balance ANOVA for Variate (Mean square) of shoot dry matter ( $\text{g plant}^{-1}$ ) of the soybean cultivars (Cultivar) under progressive soil dry water regime at observed time.

Source of variation	DF	0 DAV <sub>4</sub>	7 DAV <sub>4</sub>	12DAV <sub>4</sub>	15 DAV <sub>4</sub>	22 DAV <sub>4</sub>
Replication	2	0.67E-03	0.33	0.34	0.21	0.34
Water	1	0.22E-01	0.23E-01	2.03**	14.74**	36.28**
Cultivar	2	0.23	0.78**	0.55**	0.76*	0.90
Water x Cultivar	2	0.44E-03**	0.34E-01	0.26*	0.15	1.16
Residual	10	0.12E-01	0.34E-01	0.45E-01	0.16	0.28
Total	17	0.36E-01	0.16	0.28	1.09	5.52

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

Balance ANOVA for Variate (Mean square) of root dry matter ( $\text{g plant}^{-1}$ ) of the soybean cultivars (Cultivar) under progressive soil dry water regime at observed time.

Source of variation	DF	0 DAV <sub>4</sub>	7 DAV <sub>4</sub>	12DAV <sub>4</sub>	15 DAV <sub>4</sub>	22 DAV <sub>4</sub>
Replication	2	0.11E-01	0.11E-01	0.27E-02	0.38E-02	0.13E-01
Water	1	0.23E-02	0.42E-03	0.24E-02	0.38E-01**	0.42**
Cultivar	2	0.40E-02*	0.41E-02	0.14E-01	0.11E-01*	0.27E-01*
Water x Cultivar	2	0.94E-03	0.90E-03	0.94E-02	0.11E-02	0.17E-01*
Residual	10	0.97E-03	0.28E-02	0.33E-02	0.22E-02	0.39E-02
Total	17	0.25E-02	0.36E-02	0.51E-02	0.54E-02	0.34E-01

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

**Appendix C (Continued)**

Balance ANOVA for Variate (Mean square) of nodule dry matter ( $\text{mg plant}^{-1}$ ) of the soybean cultivars (Cultivar) under progressive soil dry water regime at observed time.

Source of variation	DF	0 DAV <sub>4</sub>	7 DAV <sub>4</sub>	12DAV <sub>4</sub>	15 DAV <sub>4</sub>	22 DAV <sub>4</sub>
Replication	2	0.19E-04	0.24E-03	0.93E-03	0.11E-02	0.22E-03
Water	1	0.86E-04	0.13E-02**	0.24E-01**	0.12**	0.34**
Cultivar	2	0.14E-02**	0.10E-02*	0.10E-02	0.46E-03	0.10E-02
Water x Cultivar	2	0.26E-03	0.74E-03	0.99E-03	0.97E-03	0.48E-03
Residual	10	0.11E-03	0.21E-03	0.77E-03	0.47E-03	0.21E-03
Total	17	0.27E-03	0.50E-03	0.22E-02	0.78E-02	0.21E01

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

Balance ANOVA for Variate (Mean square) of total number of nodule ( $\text{number plant}^{-1}$ ) of the soybean cultivars (Cultivar) under progressive soil dry water regime at observed time.

Source of variation	DF	0 DAV <sub>4</sub>	7 DAV <sub>4</sub>	12DAV <sub>4</sub>	15 DAV <sub>4</sub>	22 DAV <sub>4</sub>
Replication	2	58.10	275.60	41.52	98.67	31.19
Water	1	42.52	919.73**	2862.72**	10336.0**	25890.8**
Cultivar	2	1022.78**	465.86*	236.75	335.06**	602.23
Water x Cultivar	2	160.01	142.26*	18.50	181.89*	740.23
Residual	10	77.39	89.12	132.39	26.70	416.28
Total	17	177.07	210.55	281.19	696.13	1929.46

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

**Appendix C (Continued)**

Balance ANOVA for Variate (Mean square) of individual nodule weight (mg nodule<sup>-1</sup>) of the soybean cultivars (Cultivar) under progressive soil dry water regime at observed time.

Source of variation	DF	0 DAV <sub>4</sub>	7 DAV <sub>4</sub>	12DAV <sub>4</sub>	15 DAV <sub>4</sub>	22 DAV <sub>4</sub>
Replication	2	17.62	2.92	51.80	14.23	112.17
Water	1	1.57	5.60	239.71**	446.42**	600.62**
Cultivar	2	42.78**	4.07	59.65	32.62	27.06
Water x Cultivar	2	2.14	0.41	35.38	12.56	49.97
Residual	10	5.24	4.04	17.98	11.71	50.10
Total	17	10.54	3.81	41.95	40.13	87.06

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

Balance ANOVA for Variate (Mean square) of SPAD reading of the second most fully expanded leaf of the soybean cultivars (Cultivar) under progressive soil dry water regime at observed time.

Source of variation	DF	0 DAV <sub>4</sub>	7 DAV <sub>4</sub>	12DAV <sub>4</sub>	15 DAV <sub>4</sub>	22 DAV <sub>4</sub>
Replication	2	2.24	10.80	13.53	7.67	10.63
Water	1	0.01	2.70	40.20*	333.68**	1.00
Cultivar	2	4.85	21.68*	5.56	4.06	20.11
Water x Cultivar	2	1.79	0.45	3.22	1.68	15.08
Residual	10	1.57	4.06	6.20	9.56	10.62
Total	17	1.97	6.42	8.64	26.83	11.69

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

**Appendix C (Continued)**

Balance ANOVA for Variate (Mean square) of total N ( $\mu\text{g N g}^{-1}$  of shoot mass) in the shoot of the soybean cultivars (Cultivar) under progressive soil dry water regime at observed time.

Source of variation	DF	7 DAV <sub>4</sub>	12DAV <sub>4</sub>	15 DAV <sub>4</sub>	22 DAV <sub>4</sub>
Replication	2	1.29	0.02	0.88	0.37
Water	1	0.21	0.86*	2.92*	31.67**
Cultivar	2	0.85	0.20	1.90*	4.62
Water x Cultivar	2	3.26*	0.06	0.37	1.93
Residual	10	0.56	0.17	0.30	1.39
Total	17	0.98	0.18	0.72	3.49

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

Balance ANOVA for Variate (Mean square) of shoot dry matter, N accumulation rate and N concentration in accumulated shoot mass during 7-15 DAV<sub>4</sub> under different water regimes (Water).

Source of variation	DF	Shoot dry matter accumulation rate ( $\text{g plant}^{-1} \text{ day}^{-1}$ )	N accumulation rate ( $\mu\text{g N day}^{-1}$ )	N concentration in accumulated shoot ( $\mu\text{g N g}^{-1}$ of shoot)
Replication	2	0.15E-02	0.26	4.56
Water	1	0.17**	35.41**	40.20
Cultivar	6	0.11E-01*	0.19*	34.61
Water x Cultivar	6	0.40E-02	0.22	8.74
Residual	26	0.22E-02	0.39	18.97
Total	41	0.13E-01	2.39	19.16

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

**Appendix C (Continued)**

Balance ANOVA for Variate (Mean square) of nitrogenase activity by acetylene reduction assay ( $\mu\text{mol of C}_2\text{H}_4 \text{ hr}^{-1}\text{plant}^{-1}$ ) of nodulated root of the soybean cultivars (Cultivar) under progressive soil dry water regime at observed time.

Source of variation	DF	7 DAV <sub>4</sub>	12DAV <sub>4</sub>	15 DAV <sub>4</sub>	22 DAV <sub>4</sub>
Replication	2	4.46	4.66	9.20	9.73
Water	1	30.30*	1093.83**	4057.52**	1199.27**
Cultivar	2	28.40*	56.06**	47.31*	50.33*
Water x Cultivar	2	4.96	41.98**	40.42*	66.19**
Residual	10	4.17	2.87	9.12	8.26
Total	17	8.68	78.11	255.44	90.26

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

Balance ANOVA for Variate (Mean square) of seed yield ( $\text{g plant}^{-1}$ ) of the soybean cultivars (Cultivar) under progressive soil dry water regime at observed time.

Source of variation	DF	Seed yield ( $\text{g plant}^{-1}$ )
Replication	2	1.51
Water	1	25.49**
Cultivar	2	1.15
Water x Cultivar	2	2.02
Residual	10	0.69
Total	17	2.46

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

**Appendix C (Continued)****Experiment IV**

Balance ANOVA for Variate (Mean square) of relative water content (%) of the leaf of the soybean cultivars (Cultivar) under different soil dry water regimes at observed time.

Source of variation	DF	7 DAV <sub>4</sub>	14DAV <sub>4</sub>	4 DAR <sub>4</sub>	7 DAR <sub>4</sub>	15 DAR <sub>4</sub>
Replication	3	78.71	15.10	10.46	7.63	42.97
Water	1	823.60**	743.16**	132.11*	82.69*	361.81**
Error (a)	3	66.41	54.89	11.02	5.79	7.15
Cultivar	1	2.10	70.84	0.16	36.17	18.58
Water x Cultivar	1	0.28	33.67	0.01	17.38	16.66
Error (b)	6	21.31	21.59	10.91	7.19	10.80
Total	15	92.61	83.54	17.48	14.64	40.82

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

Balance ANOVA for Variate (Mean square) of electrolyte leakage ( $\mu\text{S cm}^{-2}$ ) of leaf tissue of the leaf of the soybean cultivars (Cultivar) under different soil dry water regimes at observed time.

Source of variation	DF	7 DAV <sub>4</sub>	14DAV <sub>4</sub>	4 DAR <sub>4</sub>	7 DAR <sub>4</sub>	15 DAR <sub>4</sub>
Replication	3	0.44	8.26	56.45	11.53	14.58
Water	1	1.82	26.52*	1061.13**	2714.41**	4349.40**
Error (a)	3	6.18	6.46	50.76	39.81	45.26
Cultivar	1	0.02	0.49	743.93*	1328.60**	976.56
Water x Cultivar	1	27.04	9.61	10.40	62.41	597.80
Error (b)	6	4.74	1.01	14.90	33.48	215.63
Total	15	5.14	6.99	198.43	297.36	493.14

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

**Appendix C (Continued)**

Balance ANOVA for Variate (Mean square) of SPAD reading of the leaf of the soybean cultivars (Cultivar) under different soil dry water regimes at observed time.

Source of variation	DF	7 DAV <sub>4</sub>	14DAV <sub>4</sub>	4 DAR <sub>4</sub>	7 DAR <sub>4</sub>	15 DAR <sub>4</sub>
Replication	3	1.44	4.23	3.62	1.66	3.56
Water	1	34.22	48.65*	6.89	21.16*	15.01
Error (a)	3	0.80	2.60	2.52	1.05	13.77
Cultivar	1	29.70	17.01	4.95	28.09*	4.95
Water x Cultivar	1	14.44	0.01	3.51	19.80	20.47
Error (b)	6	7.58	4.89	9.19	2.87	5.27
Total	15	8.71	7.69	5.93	6.29	8.27

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$

Balance ANOVA for Variate (Mean square) of yield and yield components of the soybean cultivars (Cultivar) under different water regimes.

Source of variation	DF	Pods plant <sup>-1</sup>	Seeds pod <sup>-1</sup>	100 seed weight	Seed yield
Replication	3	3.04	0.15E-01	0.19	5230.21
Water	1	49.42**	0.58E-01	0.222	420783**
Error (a)	3	3.53	0.49E-01	0.16	14784.3
Cultivar	1	0.57E-02	0.31*	104.15**	58479.6
Water x Cultivar	1	0.51	0.17*	0.59	9088.25
Error (b)	6	4.59	0.35E-01	0.22	20108.4
Total	15	7.47	0.62E-01	4.75	52330.2

\* and \*\* are significant at  $p < 0.05$  and  $p < 0.01$ .

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