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THESIS

EFFECTS OF NITROGEN FERTILIZER ON DRY MATTER ACCUMULATION, SEED YIELD AND SEED COMPOSITION OF SOYBEAN CULTIVARS

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The effect of nitrogen (N) fertilizer on the soybean cultivars was evaluated based on the physiological responses that are specific leaf weight (SLW), dry matter (DM) accumulation related to seed yield and seed composition. Field experiments were established during the wet season (June to October) and the dry season (November to March) in 2008-2009. SJ5 cultivar was grown at the National Corn and Sorghum Research Center, Thailand. Four levels of plant population density (PPD) (20, 30, 40, 60 plants m^{-2}) were assigned in the main plots and four N fertilizer rates (0, 25, 50, 75 kg ha^{-1}) were applied in the sub-plots. A greenhouse experiment was conducted hydroponically at the Department of Agronomy, Kasetsart University from September to November in 2009. Three levels of NO_3^- (5, 10, 15 m*M*) were main plots and three soybean cultivars (CKB1, SJ5, CM60) were sub-plots. Each experiment was conducted in a randomized complete block (RCB) split plot design with three replications.

In the field experiments, the importance of starter N fertilizer application in increasing seed yield was consistent in two different seasons. Relative to control (0 kg ha^{-1}), the highest N rate (75 kg ha^{-1}) had a significant greater seed yield by an average of 31 and 57% in the wet and dry season, respectively. This increase in seed yield could be due to an increase in SLW and total dry matter (TDM), which responded strongly to the N application and growth stage. The optimum PPD for maximizing seed yield was lower in the wet season (20 plants per m^{-2}) than in the dry season (30 plants per m^{-2}). Seed oil content was not significantly different among N treatments or PPD in any growing seasons. The highest N rate increased seed protein content by an average of 4% compared to the control in the wet season. However, the increment was not statistically different from the result of 50 kg ha^{-1} N treatment. In the greenhouse experiment, NO_3^- application had a significant effect on SLW and TDM, reflecting on a higher seed yield in all of the observed cultivars. The maximum seed yield was obtained from the 15 m*M* NO_3^- treatment mainly associated with the significant higher number of pod per plant. Of three cultivars, SJ5 had the highest response in TDM, leaf N content at R5 and final seed yield to NO_3^- treatment. Relative to control, 15 m*M* NO_3^- increased the seed protein content by an average of 10% but this increment was not significant different from the result of 10 m*M* NO_3^-. Seed yield and protein content was likely to be related to the extent of TDM and N content in the leaves at R5 stage of the soybean cultivars.

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Student's signature Thesis Advisor's signature

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LIST OF ABBREVIATIONS

SLW = Specific leaf weight

LWC = Leaf water content

TDM = Total dry matter

V5 = A vegetative stage of soybean plants which have 6 nodes with expanded trifoliate and unfolded leaflets.

R5 = A beginning of seed filling period of soybean plants which have approximately 3-mm long seeds in the pod at one of the four uppermost nodes on the main stem with a fully developed leaf.

R8 = A fully maturity stage of soybean plants which have 95% of the pods reaching their mature pod color.

DAF = Days after flowering

DAT = Days after transplanting

PPD = Plant population density

YFEL = Youngest fully expanded leaflet

EC = Electrical conductivity

CKB1 = Chakkrabbhandhu 1

CM60 = Chiang Mai 60

ANOVA = Analysis of variance

m*M* = Millimole

EDTA = Ethylenediamine tetraacetate

SLW-V5 = Specific leaf weight at V5 stage

SLW-R5 = Specific leaf weight at R5 stage

TDM-V5 = Total dry matter at V5 stage

TDM-R5 = Total dry matter at R5 stage

EFFECTS OF NITROGEN FERTILIZER ON DRY MATTER ACCUMULATION, SEED YIELD AND SEED COMPOSITION OF SOYBEAN CULTIVARS

INTRODUCTION

Soybean (*Glycine max* [L.] Merr.) contains a large amount of protein and lipids in the seeds, and it requires a large amount of energy (Osaki *et al*., 1988). In order to achieve high yield potential, soybean must sustain high photosynthesis rates and accumulate large amounts of nitrogen (N) in the seeds. During vegetative growth, plants have a high demand N, which sustains photosynthetic capacity, initiation and expansion of leaves and growth of stems and roots (Rufty *et al*., 1984). Biological N_2 fixation (BNF) and fertilizer N are the main sources of meeting the N requirement of high-yielding soybeans. However, BNF alone is not able to meet high N-requirement of this crop particularly under the N-deficient conditions (Hatfield *et al*., 1974; Matthews and Hayes, 1982; Thies *et al*., 1995; Wesley *et al*., 1998; Takahashi *et al*., 2006). Several researchers found an inconsistent yield response of soybean to fertilizer N (Schmitt *et al*., 2001; Gan *et al*., 2003; Barker and Sawyer, 2005).

In soybean, possible yield criteria are yield components, morphological factors that affect yield formation, and growth dynamic parameters, such as total dry matter (TDM), specific leaf weight (SLW), and light interception (LI). Since a certain TDM level is expected to optimize yield, and R5 marks the end of the period for vegetative TDM accumulation, TDM (R5) is a good putative criterion for yield (Egli *et al*., 1987). Regardless of the growth habitat, there was a linear relationship between increase in plant dry-matter increase and seed dry-matter in diverse soybean cultivars (Kakiuchi and Kobata, 2004). High SLW, the dry mass of tissue per unit leaf area, was associated with increasing leaf photosynthesis (Dornhoff and Shibles, 1970). A strong correlation ($r = 0.76^{**}$) between SLW and apparent photosynthesis (AP) which may improve seed yield in soybean (Thompson *et al*., 1995) during flowering was observed by Buttery *et al*. (1981). The correlations between AP and SLW were not consistent at pod formation or seed development stage. At the vegetative growth stage, there was a consistent tendency of AP to be positively correlated with SLW (Kokubun *et al*., 1988). Stage of development, a major physiological factor, significantly influences SLW and photosynthetic rate (Lugg and Sinclair, 1980) related to high yield.

Ray *et al*. (2005) reported that large yield responses to N fertilization were associated with greater biomass and N uptake at R5. In addition, grain yield and N content in leaves during the seed-filling period (SFP) is closely related, and the amount of N stored in the vegetative parts at seed filling stage is clearly important for development of a large seed yield (Hanway and Weber, 1971; Shibles and Sundberg, 1998). Considering seed composition, protein and oil content in the seed may be genetically controlled but those components are highly influenced by the environment (Wilcox, 1985; Burton, 1989; Egli and Bruening, 2007). Thus, numerous researchers have studied on different responses of soybean yield, seed protein, oil and other components in response to various level of added of NPK fertilizers.

Pot research in the Southeastern Plant Environment Laboratories at North Carolina State University demonstrated that supra-optimal N increased seed protein concentration of normal (NC107) and intermediate (N87-984-16) protein lines by an average of 28%, and high protein line (NC 111) by 15% (Nakasathien *et al*., 2000). This result was in agreement with that of Ohtake *et al*. (1997). In contrast, field research conducted in Kansas found that N application at the beginning pod growth stage increased soybean yield, but had no effect on grain protein or oil concentration (Wesley *et al*. 1999). Likewise, several researchers (Ham *et al*., 1975; Wesley *et al*., 1999; Starling *et al*., 2000; Schmitt *et al*., 2001) evaluating the response of soybean to N fertilization observed conflicting results and they concluded that additional research needed to be conducted to investigate the reason for the increase in yield and quality (Osborne and Riedell, 2006). Based on the previous information, the present study was developed to emphasize the effect of N fertilizer on the possible attributes related to soybean yield and quality.

OBJECTIVES

1. To observe the response of specific leaf weight (SLW), biomass distribution in each plant part at V5 and R5, seed yield and seed composition to combined effect of N application and plant population density.

2. To evaluate the effect of N fertilizer in different forms on soybean yield and seed protein content on the basis of dry matter partitioning, yield components, and N percent in seed.

3. To investigate the relationship of total dry matter (TDM) and leaf N accumulation at the beginning seed filling periods (R5) with final seed yield and protein content in soybean cultivars under the various NO_3^- solution levels.

LITERATURE REVIEW

Soybean (*Glycine max* [L.] Merr.) is one of the most valuable crops in the world not only as an oil seed crop and feed for livestock and aquaculture, but also as a good source of protein for the human diet and as a biofuel feedstock. Protein and oil from soybeans are major contributors to human nutrition either directly or through use as animal feeds. In addition to the primary seed storage compounds; protein, oil, and carbohydrate, soybeans contain a variety of minerals, vitamins, and secondary metabolites, which contribute to human health. Since its domestication around the 11th century B.C. in northeast China, soybean has been a staple food in eastern Asia (Hymowitz, 1970). Soybean has been mentioned in later records as an important cultivated legume crop throughout Asia. The world annually produced 217.6 million metric tons of soybeans in 2005-07. The top five countries; United States, Brazil, Argentina, China, and India, produce more 92% of the world's soybeans. The world average soybean yield doubled from 1.16 metric tons per ha in 1961-65 to 2.31 metric tons per ha in 2005-07 (Masuda and Goldsmith, 2009).

1. Soybean cultivation in Thailand and Myanmar

In Thailand, soybean plays an important role in social and economic aspect. Since 1983 the government has encouraged farmers to grow soybeans and has instituted a national policy to limit soybean importation. As a result, domestic production of soybean increased to approximately 670,000 metric tons in crop year 1989-1990. In the 1990-1991 crop year, however, production decreased to approximately 580,000 metric tons as a result of changes in government policy to permit free-market importation of soybean. There was a general trend of increased average yields for soybeans in the whole country from 950 kg ha^{-1} in the 1980-1990 season (Chanaseni and Kongngoen, 1992). Soybean is grown as an alternative to main crops such as maize and rice. It is cultivated in two seasons per year. The rainy season plantation occupied about 40% follows by the dry season 60%. Demand for soybean and its productions are steadily increased in the past years and were met by import (Sarobol *et al.*, 2007). Average soybean yields in Thailand are only 220-240 kg/rai (about 1.38-1.50 tons per ha), and are nearly identical to average yields from the previous decade. Corn, an alternate crop, has seen its productivity increase tremendously from about 400 kg/rai (2.5 tons per ha) in the early 1990's to currently 600-650 kg/rai (3.75-4.06 tons per ha) (Preechajarn, 2008).

In Myanmar, soybean is grown mainly in the north and south of Shan state bordering China and Thailand. It is categorized as one of the pulses while it is categorized under oilseeds in other countries. The crop is popular as a food grain at present but edible oil could be extracted from soybean. Moreover, protein-rich cake could be obtained as a by-product. It is grown in the early monsoon (wet season) in Shan state and as a cool season (dry season) crop in Central and Lower Myanmar. Based on seasonal production statistics, the area planted to soybean in the cool season accounts for 57% of the total. Under irrigation, it can produce 2.5 tons per ha and the oil and protein content of the local variety are 17-18 and 30-40%, respectively. Soybean demand in Myanmar is not only from the domestic market but also for external trade. As such, the area planted to soybean increased by 80 thousand hectares from 38 thousand in 1992-1993 to 118 thousand hectares in 2001-2002. Despite this increase in soybean sown area and production, the quantity supplied to market is still relatively low (Kyi, 2005).

2. Developmental stages of soybean

An understanding of the developmental stages of a soybean plant is important in evaluating its yield potential. The developmental stages in soybeans are characterized by the standards established by Fehr and Caviness (1977). The life cycle of soybean is divided into two stages; vegetative (V) and reproductive (R) stages.

The vegetative stages are determined by counting the number of leaves on a plant. The stages begin with VE, defined as seeding emergence, the appearance of the seedling above the soil surface. The next stage is the VC stage, which is when the cotyledonary leaf is open and the node above it has a leaf that has just unrolled. In unrolled leaf, the edges of the leaf blade must not be touching one another. Following the VC stage, all other vegetative stages are numbered according to the number of nodes on the main axis (Vn) with a fully developed leaf (Bean and Millar, 1998). A fully developed leaf is defined as one that has a leaf above them has just unrolled. Subsequent vegetative stages are defined in a similar manner. All new leaves following the unifoliate leaves will have three leaf blades and are referred to as trifoliate leaves. Trifoliate leaves appear singly on the plant at each node, with each new leaf originating on the opposite side of the stem in relation to the preceeding leaf. Beginning with the first trifoliate leaf, stages are counted consecutively with the appearance of each new leaf such as V1, V2, etc., continuing to count through the last new leaf before bloom, …Vn. When bloom begins, the V growth stages are dropped and R growth stages are started.

The reproductive stages are split into two flower stages (R1 and R2), two pod stages (R3 and R4), two seed stages (R5 and R6), and two maturity stages (R7 and R8). The R1 stage is defined as the stage at which one open flower appears at any node on the main stem. The R2 stage refers to an open flower at one of the two upper most node on the main axis with a fully developed leaf. The R3 stage is when at least one pod 5 mm (3/16 inch) long is apparent on one of the four uppermost nodes of the main stem axis with a fully developed leaf. The R4 stage occurs when at least one pod reaches 2 cm (3/4 inch) at one of the four uppermost nodes of the main stem axis with a fully developed leaf. The R5 stage occurs when at least one seed within a pod is 3 mm (1/8 inch) long at one of the four uppermost nodes of the main stem axis with a fully developed leaf. At the R6 stage, at least one seed extends to the length and width of the pod cavity at one of the four uppermost nodes of the main stem reaches its mature pod color. The R7 stage is when seed filling ends and is called physiological maturity. At this stage, the seed has about 60% moisture and contains all necessary plant parts to germinate. The normal color of a mature pod can range from tan to brown depending on the genotype. The R8 stage is when 95% of the pods have reached their mature pod color and is called harvest maturity. By this time, the soybean seed would have a moisture level of 15% which is considered the harvestable level.

3. Possible yield criteria of soybean

3.1 Specific leaf weight (SLW)

Since SLW is easy to measure on small sections of leaves, is highly correlated with net assimilation rate (NAR), and has a fairly high heritabillity, it seems the most promising characteristic for use in indirect selection for yield. Positive correlation between SLW and high NAR tended to have a high relative growth rate (RGR) (Buttery and Buzzell, 1972). Also, SLW (based on whole plant values) was highly correlated with net photosynthesis and leaf density (based on individual leaves). Dornhoff and Shibles (1970) reported an increase in net photosynthesis when pod-filling begins, and this may be a sink effect. Higher SLW may be related to higher cell surface-to-volume ratio within the leaf, and hence to a lower mesophyll resistance to CO_2 entry (Buttery and Buzzell, 1988). A positive relationship between leaf apparent photosynthesis (AP) and SLW has been observed among soybean cultivars (Dornhoff and Shibles, 1976; Bhagsari *et al*., 1977; Hesketh *et al*., 1981). Wittenbach *et al*. (1980) reported that the activity of RuBPCase varied little per unit enzyme, thus indicating that under field conditions on sunny days the quantity present would largely affect the exhibited enzyme activity. Dornhoff and Shibles (1976) found that cultivar differences in SLW were correlated with the variations in leaf thickness. They suggested that higher AP rates in leaves exhibiting greater SLW may reflect the presence of a greater quantity of photosynthetic components contained within the leaves. Wells *et al*. (1986) determined the relationship between SLW and leaf protein factors for both the field and greenhouse plants. They found highly significant positive correlations between SLW and soluble protein m^{-2} as well as RuBPCase m^{-2} in all environments. SLW (fresh weight basis) and RuBPCase activity per unit leaf area were both increased in response to progressively greater light intensity (Bowes *et al*., 1972).

For individual leaves, SLW generally decreased as leaf area increased, and then increased as leaves thickened. In most cases, leaves thickened after the period of greatest leaf area expansion. Specific leaf weight then decreased as leaves senesced. In general, the maximum SLW achieved by each leaf was successively greater with each successive node. Similarly, leaves at upper nodes were thicker than those of the lower nodes. Leaf thickening was largely the result of concurrent thickening of palisade and spongy mesophyll tissues. An important discovery was that in the uppermost thickest leaves, a third layer of palisade mesophyll cells was formed by periclinal division in the outermost palisade layer. The final SLW and leaf thickness obtained were modified by solar radiation levels during the period of leaf development (Lugg and Sinclair, 1980). Guiamet *et al.* (1986) stated that the second and fifth leaves showed larger SLW under long days (8 h-natural daylight + 8 h-low intensity artificial light + 8 h-darkness). Leaves at the fourth node from the top displayed SLW values which were similar to the top canopy position (Wesley *et al.*, 1986). Peng *et al.* (1993) demonstrated that the effect of N fertilizer on the SLW in rice was minor and inconsistent in comparison with the effects of growth stage. In general, leaf dry weight of the uppermost fully expanded leaves significantly decreased, but SLW increased as plant growth proceeded. Similarly, lack effect of N fertilizer on the SLW in sunflower was detected by Cechin and Fumis (2004).

3.2 Dry matter (DM) distribution

Soybean growth is measured by the amount of TDM accumulating in the plant. Dry weight consists of everything in the plant except water, including carbohydrates, proteins, oils and mineral nutrients. The soybean plant produces 95% of its TDM through photosynthesis (Taiz and Zeiger, 2002). Yield of a soybean crop is a function of light interception, DM production, and partition of DM into the plant's seed. The ability of individual seeds to accumulate DM is a fundamental component of the yield production process in grain crops. Determination of yield components important in yield formation helps substantiate TDM (R5) as a criterion for optimum yield. The total assimilate accumulated by the crop is called TDM, and that portion partitioned to formation of seed is called economic yield.

The term partitioning describes the distribution of the new assimilate to growth of various plant parts. The fraction, economic yield/ TDM, is termed as the harvest index (HI). The concept of HI was described as; the migration coefficient (the ratio of grain yield to the TDM at maturity) (Donald and Hamblin, 1976). Generally, a TDM (R5) of 500g m^{-2} by the beginning of the seed filling stage was required to optimize yield (Egli *et al*., 1987). Half of the dry matter produced SFP was consumed for vegetative organs and the remaining half for seed production in soybean, and the reserves stored before flowering apparently did not contribute to seed production (Kakiuchi and Kobata, 2004). In practice, higher yields of economically important crops are the result of both greater dry-matter production in leaves and increased carbon accumulation in harvestable organs (Ho, 1988). Dry matter accumulation as well as photosynthate partitioning was affected by the source of N nutrition. There was a significant increase in plant DM with fertilizer N application. However, this increase in plant DM did not result in an increase in grain yield. Furthermore, grain quality and total N uptake were not significantly affected by fertilizer N application (Diaz *et al*., 2009).

A comparison of plants of the same chronological age shows that the N_2-fixing plants accumulated less dry weight than the NO_3^--supplied plants. The decreased accumulation of dry weight was most pronounced in the roots of the nodulated plants, indicating a greater partitioning of photosynthate to the root system of the nodulated soybean plants (Finke *et al*., 1982). Total dry matter accumulation and its partitionining among plant parts were affected by N-source in both cultivars (cv. Tracy and Ransom) of soybean. Plants supplied NO_3^- accumulated more total dry matter than plants inoculated with either *Rhizobium* strain (Kerr *et al*., 1984). Nitrogen fertilization not only leads to overall increased growth and biomass production, but also results in alterations in the allocation of resources and in plant morphology. Plants growing on low NO_3^- supply typically display a higher root-to-shoot ratio than plants adequately fed with this nutrient (Van der Werf and Nagel, 1996).

3.3 Carbon (C) and N assimilation

Carbon and N assimilation are closely linked fundamental processes for plant growth. Translocation and partitioning of assimilated C and N are major determinants of plant productivity and crop yield (Gifford *et al*., 1984) because grain yield of legumes is proportional to DM and N accumulation (Muchow *et al*., 1993). The C- and N-assimilates produced are exported to the growing regions of the plant where they are incorporated into new vegetative cells and tissues, giving rise to reproductive organs and seeds, thus completing the growth cycle (Ourry *et al*., 2001). The interaction between C and N is particularly important since these elements are abundant in plants and provide the skeleton and moieties for most of the building biomolecules. From the aspect of the whole plant, inorganic N sources taken up are assimilated in roots and leaves utilizing energy and C skeletons provided by photosynthesis, which takes place in the leaves (Stitt and Krapp, 1999).

Plant leaves are the important organ for N metabolism, as well as photosynthesis. Leaves contain the complex biochemical photosynthetic machinery capable of capturing light and using the energy for the reductive assimilation of carbon dioxide (CO_2) and nitrate ions (NO_3^-) with the formation of carbohydrates and amino acids, respectively. Leaf N was correlated with photosynthetic rate (P_A), yield, and maturity (Buttery and Buzzell, 1988). Nitrogen exists in leaves primarily as ribulose biphosphate carboxylase/oxigenase and there is generally a strong relationship between N per unit leaf area and photosynthesis (Sinclair, 2004). The role of N in agricultural production is intimately connected with photosynthesis. In photosynthesis, the ‘physical energy’ of photons is converted into the ‘chemical energy’ of ATP and reduced metabolic intermediates, primarily NADPH, which are used in the synthesis of C and N assimilates of many different types, particularly carbohydrates and amino acids. These ‘fuel’ the synthesis of biochemical components of organs and ultimately provide the structure of the whole plant (Foyer *et al*., 2001). The N absorbed from roots or fixed in root nodules is transported via xylem in stems and petioles, and the N is translocated to the sink organ such as pods and seeds via phloem. Therefore, the crop should have a canopy that enables full light interception and sufficient storage of N in leaves to maintain a non-N-limited photosynthetic apparatus for converting incoming radiation into new biomass and eventually grain yield.

Soybeans redistribute significant quantities of N from their leaves and other parts of plant to the seed during reproductive growth and this redistributed N can account for from 50 to nearly 100% of the N in the seed at maturity (Hanway and Weber, 1971). During pod fill 70% of N required for seed development in legumes is derived from remobilization (Pate, 1985). The decline in the concentration of N in the leaves during reproductive growth has been related to the decline in photosynthetic activity (Boon-Long *et al*., 1983). As N is remobilized from older plant tissue to the developing seeds, senescence of plant tissue begins. During this period, it is possible that under certain climatic conditions such as high-yield environments, N supply could be limiting optimum seed production. Supplying N to the soybean plant during the time of peak seed demand may supplement existing N resources, prevent premature senescence, and increase seed yield (Nelson *et al*., 1984).

The distribution of DM and N among plant tissue is also dependent on the stage of development. Early in the plant life cycle vegetative tissues (primarily leaves) are the principal C and N sinks. The leaf is the major organ of N assimilation in many species, especially when N is plentiful (Foyer *et al*., 2001). As plants progress from early to mid to late reproductive stages the pods develop into stronger sinks for C (Scott *et al*., 1983) and N (Pate, 1985) as seeds increase their protein reserves. Structural and nonstructural carbohydrates account for approximately one third of the soybean DM. At maturity, nonstructural carbohydrates comprise 12% of the dry seed weight with the remaining carbohydrates involved in formation of cell wall material. Nonstructural carbohydrates include sucrose (41-68%), stachyose (12-35%), raffinose (5-16%), and starch (1-3%). Other low molecular weight carbohydrates, such as monosaccharides, D-pintol, D-onoitol, *myo*-inositol, galactinol and their galactosyl derivatives are present in the mature seed (Schweizer and Horman, 1981).

The processes and fluxes of N-and C-assimilates are dependent upon the supply of resources from, and conditions in, the environment. Kerr *et al*. (1984) reported that leaf total N concentration was affected by the imposed treatments in both soybean cultivars; cv. Tracy and Ransom. Leaves of NO_3^--supplied plants had the highest total N concentration. Leaf total N concentration in plants inoculated with *Rhizobium japonicum* strain 110 was similar to that in NO_3^- supplied plants, whereas N concentration in leaves of plants inoculated with *Rhizobium japonicum* strain 31 was from 12 to 27% lower than that in leaves of NO_3^--supplied plants. Taking into account the physiological changes associated with high grain yields (i.e. biomass, and N accumulation and remobilization), different N fertilization strategies may be used to increase N and C supply to the demanding grains. These strategies may be based on increasing biomass and N accumulation in vegetative tissues at R5 that would increase C and N storage for subsequent mobilization during the seed-filling period. The emerging details of C and N assimilation suggest that a regulatory system coordinates uptake and distribution of these nutrients in response to both metabolic and environmental cues (Coruzzi and Bush, 2001).

Finke *et al*. (1982) concluded that the N_2-fixing soybeans (cv. Wells) have partitioned C into production of nodular tissue and reproduction of N_2 at the expense of root and shoot growth. For young soybean plants, it is suggested that sole dependency on N_2-fixation is not only more costly in terms of N use efficiency, but also in C partitioning which could limit the potential development of photosynthate, water, and nutrient-absorbing organs. Nitrate-supplied plants invested 9% to 11% more of their net photosynthate into leaf tissue than did the N_2-fixing plants. Nitrate-supplied plants were more efficient in N assimilation (5.0 versus 8.3 mol C respired/mol N assimilated) than the N_2-fixing plants of the same age (Finke *et al*., 1982). Dry matter and N partitioning among plant tissues is affected by plant genotype, growth stage and the environmental factors. Under similar soil field conditions the fraction of above ground DM and N partitioned to leaves varied little across species of soybean (Muchow *et al*., 1993).

4. Composition of soybean seed

The economically useful part of soybean is its seed. In addition to the normal chemical constituents found in all plant tissues, seeds contain extra amounts of substances stored as a source of food reserves to support early seedling growth. These are principally carbohydrates, fats and oils, and proteins. The major composition of seeds is determined ultimately by genetic factors and hence varies widely among species and their varieties and cultivars. Some modifications of composition may result from agronomic practices (e.g., nitrogen fertilizer application, planting dates) or may be imposed by environmental conditions prevalent during seed development and maturation; but such changes are usually relatively minor (Bewley and Black, 1994). Soybean seeds are composed of approximately 20% oil, 36% protein, 30% carbohydrate, 9% crude fiber, and 5% ash (Padgette *et al*., 1996).

4.1 Seed protein

The harvested seed of soybean has a high protein content (380 g kg^{-1}) apart from carbohydrates (380 g kg^{-1}) and oil (200 g kg^{-1}) (Bewley and Black, 1994). The bulk of the proteins in soybean seeds are stored in protein bodies which may vary from 2 to 20 µm in diameter. Seed proteins can be broadly classified into two categories; viz. housekeeping proteins and storage proteins. The housekeeping proteins are responsible for maintaining normal cell metabolism. A more recent classification divides these proteins into storage, structural and biologically active proteins. The major biologically active proteins include lectins, enzymes and enzyme inhibitors. These are minor proteins and may have nutritionally more balanced amino acid composition than storage proteins.

Based on biological function in plants, seed proteins are of two types: metabolic proteins and storage proteins. Metabolic proteins include enzymatic and structural, and are concerned in normal cellular activities, including the synthesis of the second type. Storage proteins, together with reserves of oils, are synthesized during soybean seed development. Following seed germination they provide a source of N and C skeletons for the developing seedling. The majority of soybean protein is storage protein. Based on solubility patterns, legume seed proteins are divided into albumins and globulins. Albumins are soluble in water, whereas globulins are soluble in a salt solution. Under this classification system, most soy protein is globulin. Globulins in numerous legume species are further divided into two distinct types: legumin and vicilin. Compared with vicilins, legumins have larger molecular size, less solubility in salt solutions, and higher thermal stability. In addition, certain soy proteins have their trivial names. For example, the two types of legume globulins, legumins and vicilins, are commonly known as glycinin and conglycinin in soybeans, respectively. These common names are apparently derived from the genus name of soybean plant, *Glycine* (Nielsen, 1985a).

The seed storage proteins on the other hand, are non-enzymatic and have the sole purpose of providing proteins (nitrogen and sulfur source) required during germination and establishment of a new plant. Soy protein is generally regarded as the storage protein held in discrete particles called protein bodies, which are estimated to contain at least 60-70% of the total soybean protein. Upon germination of the soybean, the protein will be digested, and the released amino acids will be transported to locations of seedling growth. The seed storage proteins for the purpose of classification can be divided into the major solubility classes of albumins, globulins, prolamins and glutelins, which are soluble in water, salt, aqueous alcohol and acid or alkali solutions, respectively. Legume proteins, such as soy and pulses, belong to the globulin family of seed storage proteins called leguminins (11S) and vicilins (7S), or in the case of soybeans, glycinin (11S globulin) and β-conglycinin (7S globulin) (Derbyshire *et al*., 1976).

Nitrogen is biologically combined with C, H, O, and S to create amino acids, which are the building blocks of proteins. Amino acids are used in forming protoplasm, the site for cell division and thus for plant growth and development. Nitrogen is a component of proteins, and proteins form the photosynthetic apparatus that mediates both the “light” and the “dark” reactions. The former reaction includes proteins involved in light harvesting, electron transport and photo-phosphorylation, whereas the latter reaction includes proteins involved in CO_2 assimilation, photorespiration, and carbohydrate production. Nitrogen application elevated the protein content but lowered that of the sulfur amino acids. The major storage proteins are the globular 11S glycinins and 7S conglycinins which together account for over 70% of the total seed protein (Thanh and Shibasaki, 1978). Relative accumulation of glycinins and β-coglycinins affects the nutritional quality of the seed protein and characteristics important in the production of foods (Adachi *et al*., 2004).

Seed protein concentration is an inherited trait influenced by the environment (Burton *et al*., 1989) and may depend less on the genotype of the embryo than on that of the plant on which the seeds develop (Singh and Hadley, 1968). Thus, whole-plant processes (e.g., carbon (C) and nitrogen (N) fixation and allocation), biochemical control (e.g., hormonal regulation), or environmental effects (e.g., nutrient availability) may interact to determine the nutrient composition and yield of soybean seeds. Hayati *et al*. (1995) suggested that, ignoring genetic differences in seed N composition, seed N ‘demand’ may not exist; instead, seed N concentration may be mainly determined by the supply of N from the plant, not by the characteristics of the seed.

4.2 Lipids

Soybean lipids are deposited in spherosomes which have been identified by electron microscopy. Spherosomes in soybean cotyledons are interspersed between protein bodies. Neutral lipids primarily consist of triglycerides, accompanied by smaller proportions of free fatty acids, sterols, and sterol esters. The main components in neutral lipids, phospholipids, and glycolipids are palmitic, oleic, linoleic, and linolenic acids. Soybean oil contains about 300 to 400 mg of plant sterols per 100 g. Major components of soy sterols are sitosterol (53 to 56%), campesterol (20 to 23%) an, and stigmasterorol (17 to 21%). Soybean oil contains five commercially important fatty acids: palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic

(18:3). The relative content of fatty acids influences the physical and chemical characteristics of the oil thus the suitability of the oil for a particular use. Soybean lines are currently being developed to express amended fatty acids thus increasing potential uses of the oil (Spencer *et al*., 2004).

Study on the lipid composition of developing soybeans (Privett *et al*., 1973) revealed that in the early stages of development of the bean, the lipid was virtually devoid of triglyceride and the major constituents consisted of glycolipids and phospholipids. As the bean developed, there was a rapid synthesis of triglyceride that paralleled the deposition of lipid. Simultaneously, unknown substances which occurred in relatively large amounts in the neutral, as well as the glycolipid and phospholipid, fractions of the immature bean diminished to less than 2% of the total lipid in the mature bean. The glycolipids and phospholipids also increased as the bean developed but at a much slower rate than the triglycerides and became minor components in the mature bean. The major component of the phospholipids in the mature bean was phosphatidic acid. It decreased as the phosphatidyl choline, phosphatidyl ethanolamine, and phosphatidyl inositol, as well as triglyceride, increased. During soybean seed development the rate of lipid biosynthesis per seed increases markedly, resulting in a mature seed containing 20 to 25% lipid by weight. As the seed reaches maturity, lipid synthesis declines. Between 15 and 45 DAF, the rate of fatty acid accumulation per seed increases 10 to 20 fold. Most of the lipid in soybean seeds is stored in the cotyledons in the form of triglycerides packaged in specialized structures, often referred to as oil bodies or spherosomes (Ohlrogge *et al*., 1983).

Oil is located in smaller structures called spherosomes which are interspersed between the protein bodies and are 0.2 to 0.5 µm in diameter. The oil normally contains approximately 10% linolenic, 55% oleic, and 30% linoleic acid, with up to 50% variation in a specific component. Seed lipids are stored in the form of triacylglycerol (TAG) which fatty acids are esterified to glycerolipids. Fatty acids vary in type and content according to genetic and environmental factors (Kinney *et al*., 1994), which affect their nutritional value and processing property. The lipids in soybean seeds typically contain 10% linolenic acid, which is a polyunsaturated fatty acid with one more double bond than linoleic acid. Linolenic acid is considered unstable and responsible for the development of off-flavors because it is easily oxidized compared to other unsaturated fatty acids (Frankel *et al*., 1980). In the oil industry, the oxidative stability of edible oil is improved by hydrogenating soybean oil to reduce linolenic acid levels; however, *trans*-isomer fatty acids are produced during hydrogenation, which are believed to increase the risk of heart disease.

The major factor limiting the use of soybean protein products is their off-flavor. The presence of significant amount of polyunsaturated fatty acids and the enzyme lipoxygenase are responsible for off-flavors and off-aroma compounds at various stages of processing. During processing, components extracted from soybeans by organic solvents such as hexane are classified as crude oil. Major components of crude oil are triglycerides. Commercially available crude soy lecithin contains various proportions of neutral and polar lipids. Neutral lipids are mainly triglycerides, whereas polar lipids consist of phospholipids and glycolipids. Minor components include phospholipids, un-saponifiable material, free fatty acids, and trace metals. The concentrations of these minor compounds are reduced after typical oil processing. Refined soybean oil contains more than 99% triglycerides. Triglycerides are neutral lipids, each consisting of three fatty acids and one glycerol that link the three acids. The functional properties, oxidative stability, as well as the nutritional value of edible oils in general and soybean oil in particular are all determined by their fatty acid composition, geometric configuration, and positional distribution.

In soybean, oil, sugars and other nonprotein components are most affected by changes in protein content. When protein content is increased, there is a significant decrease in nonprotein constituents such as oil, sugar, and pentosans (Krober and Cartter, 1962). The lipid content of soybeans and the composition of their fatty acid are influenced by genetic as well as environmental factors. The fatty acid composition of soybean oil is influenced by the genetic character and climatic conditions during which oil is elaborated. The average oil content on a moisture-free basis in soybean seed is about 20%. However, temperature has a marked effect on both polyunsaturated fatty acids and the oil content of soybean. Under controlled temperature conditions, soybeans produced oil contents of 23.2% at 29ºC, 20.8% at 25ºC, and 19.5% at 21ºC (Wolf and Cowan, 1975).

5. Nitrogen requirement of soybean

Nitrogen is required by plants in greater quantities than any other mineral element. Much of this high demand reflects the large amount of N invested in the photosynthetic apparatus (Kumar *et al.*, 2002). The availability of N is thus a significant determinant of both photosynthetic capacity and crop yield. The sources available to plants are usually inorganic forms such as NO_3^- and NH_4^+. Their availability changes unexpectedly and rapidly in a natural environment, and limits plant growth and development. Plants can absorb and assimilate various forms of N, though high amounts of added nitrate (NO_3^-) and the presence of nitrifying bacteria mean that NO_3^- is the principal form available to the roots of crop plants in agricultural conditions. The N source functions not only as a substrate for the assimilation, but also as a signal for growth and development by regulating gene expression and thereby metabolism. In higher plants, NO_3^- can be reductively assimilated in both roots and shoots. Application of NO_3^- fertilizer leads to a stimulation of all the steps in the pathway of N assimilation. This results in increases in NO_3^-, ammonium, amino acids, proteins and other N-containing constituents in the plant (Scheible *et al.*, 199b). The pronounced modifications in metabolism and development that result from quantitative and qualitative changes in the availability of N are either a consequence of N assimilation or are due to signaling by either NO_3^- or by metabolites that are downstream of NO_3^- assimilation. It has long been suspected that NO_3^- is not only a resource but that it also acts, directly or indirectly, to trigger signals that modulate gene expression, metabolism and development (Redinbaugh and Campbell, 1991).

Since N is directly involved in the photosynthesis process, soybean requires a large amount of N relative to other crops. Nitrogen is integral component of many compounds, including chlorophyll and enzymes, as well as amino acids related proteins. The N requirements of pulses and soybeans were so great that sustained seed growth demanded continued N translocation from vegetative tissues, which must eventually induce senescence in these tissues, restrict duration of the seed-fill period, and limit seed yield. Soybean demand for N is the highest among agronomic crops (Sinclair and de Wit, 1975). It can exceed 92 g kg^{-1} seed for optimum seed yield (Flannery, 1986). The N required for plant growth can be obtained either from symbiotic fixation of atmospheric nitrogen (N_2) or from the direct uptake of soil inorganic N. It has the capacity to derive a considerable proportion of its N requirement from the atmosphere through symbiosis with *Rhizobium* and environment interactions (Ham and Caldwel, 1978). Soybean uses BNF to produce approximately half of its total N requirement (Harper, 1987). The remaining N is derived from soil inorganic N, mineralized organic matter, or residual N from the previous crop. Soil NO_3^- is the main N source utilized up to the beginning pod growth stage (R3) (Pedersen, 2004), with crop use depleting soil inorganic N. Nitrate utilization and NO_3^- reductase activity drop rapidly at this time (Shibles, 1998).

In most situations the amount of N fixed by soybean is not enough to replace N export from the field with grain, or is at best close to neutral if N from below-ground parts is included. It is clear from the reviewed data that high yielding soybean requires large amounts of N to support both above-ground biomass and high protein seed (Salvagiotti *et al*., 2008). Soil nitrate is the main N source utilized up to the beginning pod growth stage (R3) (Pedersen, 2004), with crop use depleting soil inorganic N. Although the soybean is high in protein and requires large amounts of N, N fertilizers are not used extensively on this crop. Tanner and Hume (1978) stated that there are two stages which have been reported to respond to more N fertilizer application. The first is during 2-3 weeks after sowing. Usually nodules are formed by 15-20 days after sowing and commence to fix N at the 20-30 days after sowing. Thus, in low N soils a basal nitrogen application is essential for high rates of photosynthesis and growth at seeding stage. Sole N_2 fixation is generally insufficient to support vigorous growth of shoot and roots, which results in the reduction of plant growth and seed yield. The plant N requirement may not be met during early vegetative and later productive phases by N_2 fixation. Symbiotic N_2 fixation begins only after nodule formation, which is proceeded by the colonization of the rhizosphere and the infection of legume roots by rhizobia (Hardy *et al*., 1971). Maximum BNF occurs between the R3 and R5 stages of soybean development (Zapata *et al*., 1987), and any gaps between crop N demand and N supply by BNF must be met by N uptake from other sources.

Nitrogen fixation by grain legumes generally reaches a peak at early podfill and then declines at the late reproductive phase (Imsande, 1988; Latimore *et al*., 1977). Thus, mineral N may be a critical source of N for grain legumes during both the early vegetative and late reproductive periods. In the absence of N fertilizer, soybean seedlings are dependent on cotyledonary N during the critical establishment phase. Soybean plants typically experience a transient N stress as the cotyledonary reserves are depleting, which extends until development of the N_2-fixation system 3.5 to 4 weeks after germination (Israel, 1981). Soybean plants totally deprived of any starter N source undergo a severe N stress period following depletion of cotyledonary N reserves and before functional nodule development occurs (Matthews and Hayes, 1982). Nitrogen fixation will increase in high yielding environments since the nitrogenase, located in the nodules, will adjust its activity to the demand of the legume (van Kessel and Hartley, 2000). In high yielding conditions, the N supply may not be enough to achieve maximum grain yield because the N uptake rate during the seed filling period may be insufficient to fulfill the increasing demand of the growing seed. Increasing the supply of N to plants leads to increased growth, accelerated germination of seeds, and morphological changes such as decreased root: shoot ratios, root architecture, delayed flowering, tuber initiation and senescence (Stitt and Krapp, 1999).

6. Effect of plant population on soybean

6.1 Yield components and seed yield

Optimum plant population is an important factor that determines the yield of the soybean crop. Optimum population is the minimum population required by the crop to produce maximum yield. Plant populations and row spacing can affect leaf area and yield. Determining the minimum population required to reach an optimum LAI and maximize yield for a specific environment is an economically important decision because seed cost can affect soybean profitability (Boquet, 1990). Response to either increase in population or narrow row spacing is closely correlated to the ability of the crop to intercept most of the incoming solar radiation without excessive growth that lodging occurs (Minor, 1976).

Early work by Shibles and Weber (1966) in Iowa showed that the rate of dry matter accumulation, a direct measure of vegetative growth, was linearly related to the percent interception of incoming solar radiation. They found that a LAI of approximately 3.2 was required to achieve 95% light interception and 95% of maximum dry matter production. Light interception of 95% is important because it is the value at which the crop should theoretically achieve canopy closure, maximum canopy photosynthesis for the developmental stage, and maximum yield for the environmental conditions. Herbert and Litchfield (1984) used growth analysis techniques to look at the physiological basis of increased seed yield among narrow rows and varying populations. Seed yield was increased 31% and 16% by decreasing the row width from 75 to 25 cm and 50 to 25 cm, respectively. They determined that narrow rows (25 cm) with higher densities (80 seeds m^{-2}) produced both higher LAI and more dry matter than narrow rows with lower densities (25 seeds m^{-2}), regardless of the yield loss that occurred from lodging at the higher densities. The increase in biomass production from the highly populated, narrow-row soybean occurred due to the timely closure of crop canopy that maximized light interception.

Although many studies have been conducted to determine the effect of narrow vs. wide rows on soybean yield, little information is available explaining why these yield responses occur. Board *et al.* (1990) conducted to determine the roles of reproductive partitioning, light interception duration (LID), and total dry matter duration (TDMD) between emergence to R5 (E-R5) and R5 to R8 on narrow-row seed-yield enhancement. Their results indicated that, at late plantings, both narrow-row enhanced LID (E-R5) and LID (R5-R7) play a role in the seed yield increases that occur at narrow compared with wide rows. Hatam and Munir (1994) evaluated the effect of planting time and population on growth and grain yield of soybean. These results indicated that plant population density had no significant effect on the number of pods on the mainstem as well as the branches. However, increasing plant population density (PPD) from 333 to 1001 thousand plants ha^{-1} slightly reduced yield components such as pods/mainstem and branches, and 100 grain weight. This reduction was compensated by the increasing plant population density and the grain yield steadily increased with increase in plant population density. Ethredge *et al.* (1989) conducted field experiments to determine the influence of row spacing, plant population, and cultivar on yield components of soybean. They revealed that the number of pods per plant was not affected by row spacing, but decreased significantly with increasing plant population. This decrease in pod number per plant with increasing plant population can occur without a change in yield per hectare because more plants would be present.

Boquet (1990) examined the effects of PPD on determinate soybean planted at two post-optimal planting dates. The results showed that plant growth, particularly branch development, was not influenced by intrarow plant competition at the lower PPD whereas at higher PPD, plant components were increasingly reduced by intrarow plant competition. Large reductive development resulting from late vegetative and reproductive development resulting from late planting and/or increased PPD severely restricted the ability of determinate soybean to respond to increase in PPD with yield increase. Norsworthy and Shipe (2005) found that the largest portion of seed yield from wide-row soybean was generally contributed by the branch fraction, regardless of genotype. Since the seeding rates for wide-row (97 cm) soybean are less than those of narrow-row (19 cm) soybean, the ability of soybean to branch is greater in wide rows. Conversely, in narrow-rows, main stem yield is the primary contributor to total yield. Therefore, they suggested that mainstem yield should be used as criteria for selecting superior soybean genotypes for narrow rows.

Holshouser and Whittaker (2002) detected the effects of plant population and row-spacing on early soybean production systems in the Mid-Atlantic USA. They found that with little drought stress, no response of soybean yield to plant population occurred. A population of 208,000 plants ha^{-1} was adequate for maximum yield at a site having only a brief period of stress; populations over 600,000 plants ha^{-1} were required to maximize yield where drought stress limited leaf area production. Gan *et al*. (2002) studied physiological response of soybean genotypes to plant density. They observed that total biomass, plant N derived from N_2 fixation and seed yield of all three genotypes (determinate; semi-determinate and indeterminate) responded positively to increased plant density. Double density significantly increased seed yield per unit of area, by 93, 37 and 43% for the determinate Wuyin9, the semi-determinate You91-19 and the indeterminate Jufeng, respectively.

The greenhouse experiments gave results similar to those obtained in the field. For all genotypes, yield per unit of area was higher at double than at single density. The authors suggested that a program for high soybean grain yields should employ high plant population density for both determinate and indeterminate genotypes. Ono (1985) conducted an experiment on two high yielding soybean varieties, AGS 154 and SJ-5, with different within-row spacing and plants per hill. He detected that spacing within-row and the number of plants per hill significantly affected 100-seed weight. The maximum 100-seed weight was achieved in the treatment with wider within-row spacing and less plant density. This increase in 100-seed weight was also reported by Banset *et al*. (1974), who found that seed weight affected by within-row spacing. As seed yield, plant height and number of seeds per pod in soybean were not significantly influenced by either spacing within-row or the number of plants per hill, but number of branches per plant and pods per plant were significantly differences.

6.2 Seed quality

Ideal soybean production systems achieve both high seed yield and high concentrations of desired seed quality components. However, the relationships between seed quality and yield of soybean are largely unknown (Yin and Vyn, 2005). Many studies have been conducted to evaluate the effects of row spacing on soybean yield, but few have reported on protein concentration. Pedersen (2005) evaluated physiological response of soybean to plant spacing and expected that narrow row spacing and increased plant population would improve light interception and that would then have a cascade effect, increasing crop growth rate and potentially improving seed production.

Al-Tawaha and Seguin (2006) studied seed yield, oil and crude protein (CP) concentrations effected by row spacing, seeding date and weed. Based on their results it was evident that year, seeding date, and weeds affected total and individual isoflavone concentrations, while row spacing had no effect. Year, row spacing, and weeds significantly affected seed yields. Boydak *et al.* (2002) stated that oil and protein contents were both affected by year. Row spacing and irrigation affected protein and oil contents significantly in both years. Row spacing of 70 cm had the highest protein content, followed by spacing of 60, 40, and 50 cm, respectively.

7. Effect of N fertilizer on soybean

7.1 Biological nitrogen fixation

On average, 50-60% of soybean N demand is met by BNF across a wide range of yield levels and environments and the proportion of plant N derived from fixation decreases with increasing inputs of N fertilizer. The inhibitory effects of externally supplied N especially NO_3^- reviewed by Streeter (1988) have been suggested that there are multiple effects of nitrate inhibition, such as the decrease in nodule number, nodule mass, and N_2 fixation activity, as well as the acceleration of nodule senescence or disintegration. The effects of nitrate on nodule formation and growth are influenced by nitrate concentration, placement and treatment period as well as legume species (Gibson and Harper, 1985).

Vigue *et al.* (1977) determined the effects of urea- and nitrate-N sources on nodulation of soybeans. They observed that urea allowed effective nodule development and function, as evidenced by nodule mass, acetylene reduction, and difference between the total Kjeldahl N gain per plant and urea uptake measurements.

Plants grown in solutions with as high as 18 m*M* urea-N produced nodules capable of N_2 fixation (C_2H_2 reduction assay) while NO_3^- concentrations low as 2 m*M* inhibited nodulation. The amount of N fixed by nodules of plants grown on urea varied from 27 to 71% of total plant N, depending on urea concentrations of the nutrient solutions. However, a heavy supply of N fertilizer often depresses nodule development and N_2 fixation activity and induces nodule senescence, which also results in the no-effect or sometimes in reduction of seed yield (Takahashi *et al*., 2006). Hinson (1975) also stated that the amount of soil N causing the reduction in N fixation varies in relation to the soil and climatic conditions of the environment. Whereas early application of even small amount of N often results in temporary suppression of nodule establishment and subsequent activity, an early-season N-deficiency may delay early crop growth and thus the development of an efficient nodulation system.

Some scientists argue that small amounts of fertilizer N are needed at the time of planting as this can stimulate plant growth prior to active nodulation and N fixation (Harper and Cooper, 1971; Hatfield *et al*., 1974). The greenhouse research utilizing sand or solution culture has demonstrated the importance of the presence of some soil or fertilizer N for initial growth of soybean, even in the presence of adequate nodulation (Hatfield *et al*., 1974). Wych and Rains (1979) reported that a small amount of starter N has a synergistic effect on subsequent plant growth and increased N assimilation by soybean plants. Applying fertilizer-N has been proposed as an aid for increasing available N in the soil. Nodulated soybean plant can assimilate combined N forms such as nitrate or ammonium nitrogen in addition to its capability to fix atmospheric N (Oyama and Kumazawa, 1979). Studies of nodulated soybeans showed significant yield response to frequent N additions when the N_2 fixation apparatus could not meet N demand (Thies *et al*., 1995). Nitrogen-fixing capacity begins to decline after the R5 growth stage, which also coincides with the time of peak N demand (Shibles, 1998). However, studies with N applied during reproductive stages have generated inconsistent results. Nitrogen applied at R3 to R4 stages was suggested to significantly increase grain yield with high-yield-potential irrigated soybean (Wesley *et al*., 1998).

Afza *et al*. (1987) conducted a study with N application during the R4 growth stage. The plants translocated 40 to 67 % of applied N into the pods and resulted in a significant seed yield increase. Results also showed that lower N rates (less than 40 kg N ha^{-1}) did not inhibit N_2 fixation. Report by Jones *et al*. (1981) indicated that soybean grown in solution culture supplied with mineral N assimilates substantially more N than do nodulated plants. In pot and solution culture studies it has been found that suppression of N_2 fixation by N application results in greater allocation of N above ground (George and Singleton, 1992). Although BNF is the most sustainable and lowest cost source of N, and in many cases there is no response to added N, it would be profitable to apply additional N in some cases. Well nodulated soybeans are most likely to respond to N fertilization when grain yields are above 4.5 Mg ha^{-1}, and hence, an additional N uptake to support this increase is expected. A larger N fertilizer rate will be needed to: i) provide N that would have been fixed but which has been suppressed by N fertilization, and ii) obtain the additional N that is required to actually increase grain yield (Salvagiotti *et al*., 2008).

7.2 Yield components and seed yield

Nitrogen applications increased plant N concentration and plant N at R6 but did not increase plant DM, grain N concentration and removal, grain yield, or grain quality components. Nitrogen, as urea, was not an effective soybean fertilization practice when applied at the beginning pod stage across Iowa’s predominant soybean production areas and typical midsummer environmental conditions. Use of slow-release coated urea and concentrating N into bands between alternate rows did not enhance response to N application. Results from this study lead to the conclusion that growers should not consider fertilizer N applied to soil during early reproductive stages as a method to increase soybean grain yield and quality (Barker and Sawyer, 2005). The greenhouse study by Yoshida (1979) utilized five N rates from 15 to 150 mg kg^{-1} NO_3^--N in complete hydroponic solutions and a control containing no N. The author concluded that total seed yield increased from 0.9 g with the control to 81.6 g per pot with 50 mg kg^{-1} N while the highest N rate, 150 mg kg^{-1}, yielded 76.7 g per pot.

Macronutrient research has shown that supplemental applications of N have increased seed yield in some studies (Wesley *et al*., 1998; Purcell and King, 1996). A study conducted in Kansas with irrigated soybean found significant soybean yield response when N was applied between the R3 and full pod stage (R4) (Wesley *et al*., 1998). Gascho (1993) in Georgia studied a variety of N application treatments on irrigated soybean with high yield potential (3360- 3695 kg ha^{-1}) at the R3 to R5 growth stages. Nitrogen increased yields by more than 340 kg ha^{-1} on sandy soils and up to 340 kg ha^{-1} on loamy soils over a period of 5 yr. Osborne *et al*. (2001) recommended that small differences in yield and quality may not be sufficient to offset additional fertilizer cost if starter fertilizer is not in the current management technique. Conversely, Cattlelan and Hungria (1994) reported that under normal conditions the application of this basal N does not lead to increased yield. When soil or fertilizer nitrogen is utilized, symbiotic fixation is reduced correspondingly. Starling *et al*. (1998) proposed an at-planting application of N could be used to promote early vegetative growth in late-planted systems. They found that seed yield of all three genotypes (Cook, AU86-23970D, AU86-23971I) was increased by nitrogen application, on average by at least 8%.

Ham *et al*. (1975) cited that although results from field studies on the effect of N fertilizer on soybean yield have been mixed, many studies have shown an increase in yield and associated dry matter accumulation as a result of N application to soybean. Taylor *et al*. (2005) conducted a field study to determine the optimum economic rate of N that would stimulate early dry matter accumulation, and thus yield, in late-planted soybean. They suggested that N applied at planting to late-planted soybean in the Deep South is warranted. N decreases soybean nodulation in terms of mass and numbers, but greater nodulation at lower N rates did not compensate for N added as fertilizer. The results of this experiment showed that nitrogen application increased seed yield, with a steady yield increase up to an N rate of 75 kg ha^{-1}, regardless of planting date, cultivar, or environment.

Xuewen (1990) studied whether N application would increase soybean yield and also indicated that N application can promote plant growth, enhance the accumulation of dry matter/plant and improve some agronomic characters such as pods/plant and seed/pod, thereby increasing seed yield. N levels of 30 and 60 kg ha^{-1} significantly increased yield. Imsande (1989) reevaluated the effect of rapid N_2 fixation during pod fill on soybean seed yield. The data in this review indicated that N_2 fixation increases mean seed weight, because of the strong positive correlation found between mean seed weight and seed yield, enhanced N_2 fixation during pod fill will significantly increase the seed yield of field-grown soybean. On the other hand, Ham *et al*. (1975) reported that the yields of the nonnodulated isoline usually respond strongly to added fertilizer N, whereas seed yields of nodulated soybean grown on fertile soil are seldom increased significantly by the addition of fertilizer N. Gan and Rerkasem (1992) determined that a minimum of 25 kg N ha^{-1} starter N application was insufficient for maximizing both total and marketable pod yield as well as total amount of nitrogen fixed. Cattenlan and Hungria (1994) also stated that in highly N-deficient soils or when plant residue in the soil has a high C: N ratio, small quantities of N may be required to sustain vigorous plant growth until the exponential phase of N_2-fixation begins. However, no more than 20 kg of N ha^{-1} is recommended.

7.3 Seed composition

7.3.1 Seed protein content

Soybeans are crops with a low seed production efficiency compared with other cereal crops; in soybean, 2 g of photosynthate is needed to produce 1 g of seed while in cereal crops only 1.3-1.4 g is needed according to the calculations based on conversion of chemical components (Sinclair and de Wit, 1975). Soybean seeds contain approximately 400 g kg^{-1} protein at maturity; consequently the accumulation and metabolism of N-containing compounds are important aspects of seed development. Nitrogen is supplied to the fruit as a variety of amino acids (Rainbird *et al*., 1984). Productivity and economic value of soybean are related to accumulation of N and protein in the seed. Brim and Burton (1979) increased protein concentration in one population through six cycles of recurrent S1 family selection without reducing seed yield. Accumulation of the seed components is influenced by genetics and environmental factors (Hoeck *et al*., 2000).

To achieve high yield potential or high quality, soybean must accumulate large amounts of N in seeds and requires a large amount of energy provided by N application. A pot experiment shows that R-plants (Rhizobia) accumulated consistently low N content in leaves, stems and roots as compared with N and (N + R) plants. Combined N can entirely replace the fixed N in the N nutrition of soybeans. N-plants give highest protein content in pods per plant and the opposite was found true concerning R-plants. The efficiency of pod protein production of the plants fed with combined N was increased when they were shaded (Rabie, 1986). Nitrogen limitation decreases the gas exchange capacity of plants that is indicative of decreased carboxylation capacity and decreased ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco) activity and/or protein (Brown, 1978). Notably, the enzyme Rubisco itself accounts for more than 50% of leaf N in C_3 plants (Kumar *et al*., 2002).

Early work by Castache and Nica (1968) found that protein content was increased by N and P, and oil content increased and decreased by P and N, respectively. Soybean plants responded to supplementary N application, producing seeds of greater weight and protein content. These results differ from that of Chesney (1973) who reported that protein in the soybean seed (avg. 34.6%) was not significantly influenced by N, P, or K, and there were also no significant interactions. Other workers have also shown that seed N or protein concentrations vary depending on N availability (Streeter, 1978). Enhancing nutritional value of seed protein involves increasing total protein, enhancing content of particular subunits, increasing specific amino acids, in particular those that are limiting in monogastric nutrition, and minimizing the proteins that have been shown to have deleterious effects. Seed N concentration was reduced on plants that could not increase N uptake or fixation (i.e. non-nodulated plants with NO_3^- removed from the culture media). Enhancing nutritional value of seed protein involves increasing total protein, enhancing content of particular subunits, increasing specific amino acids, in particular those that are limiting in monogastric nutrition, and minimizing the proteins that have been shown to have deleterious effects.

Ham *et a*l. (1975) noted that N fertilization increased seed yield, weight per seed, seed protein percentage, and kg protein/ha. Chin (1981) also observed that high protein content in seeds is an attribute of seed quality which can be improved by supplementary N. Schmittt *et al.* (2001) evaluated in-season N applications on soybean in Minnesota. Treatments of urea and polycoated urea applied broadcast and in subsurface bands increased soil NO_3^- (0 to 30 cm depth) at the full seed stage (R6). Nitrogen did not increase grain yields but slightly increased seed N removal and grain protein. Nakasathien *et al.* (2000) suggested that a cultivar with a regular concentration of protein in the seed had a biochemical capability of synthesizing more protein when N was available. Thus, accrual of soybean proteins is influenced by both genetics and the environmental conditions. Also, hydroponics experiments conducted by Peak *et al.* (1997) have shown external N sources to increase soybean protein concentration. Soybean plants dependent upon N fixation yielded seeds with a protein concentration of 35% while those supplemented with 6 m*M* KNO_3 produced seeds containing 41% protein. These experiments indicate the potential for increasing protein quantity by increasing N availability to the plant.

Ohtake *et al.* (1997) stated that N regulation of storage protein subunit levels of soybean seeds was evaluated using non-nodulated isoline (T201) and nodulated isoline (T202) with solution culture in the greenhouse. The results indicated that non-nodulated (T201) has a normal, non-defective, β-subunit gene, and that limited N availability decreases accumulation of β-conglycinin in soybean seeds irrespective of whether N was derived from N_2 fixation or from NO_3^- absorption. The concentration of glycinin was relatively constant irrespective of total N concentration of seeds. On the other hand, the accumulation of β-conglycinin especially the β-subunit was significantly influenced by N concentrations. This was the first discovery of N regulation of soybean storage protein accumulation. This result was confirmed by following researchers (Paek *et al.*, 1997, Nakasathien, 2000).

7.3.2 Seed oil content

The obstacle to increase the protein and oil contents of commercial cultivars is the inverse and consistent relationship among the productivity, protein concentration and the negative correlation between protein and oil concentration in the soybean seeds (Burton, 1985). There are evidences that the growth and composition of soybean seeds are controlled by the quantity of nutrients, made available by the genetic of the mother plant or the environment.

Osborne *et al*. (2002) investigated the effect of starter fertilizer N rates and sources on soybean yield, protein, and oil content in the cool soils of the Northern Great Plains. They recommended that applying N as starter has the potential to increase soybean yield and quality when soil temperatures are cool. However, Barker and Sawyer (2005) recommended that growers should not consider fertilizer N applied to soil during early reproductive stages as a method to increase soybean yield or grain quality. Similarly, in field experiments, application of N at various growth stages has not proven effective in improving the protein or oil concentration of soybeans (Schmitt *et al*., 2001). Haq and Mallarino (2001) determined fertilization effects on soybean grain oil and protein concentrations in 112 field trials because numerous studies investigated fertilization effects on soybean grain yield, but few focused on oil and protein concentrations. They summarized that although increase of grain oil and protein concentrations from fertilization are unlikely, a yield response to fertilization will not result in significant concentration decreases and will increase total oil and protein production. Grain protein, oil and fiber concentration were the same with or without N application.

MATERIALS AND METHODS

There are three experiments in this study; two field experiments and a greenhouse experiment. Field experiments were conducted at the National Corn and Sorghum Research Center, Nakhon Ratchasima, Thailand during the wet season (June to October) as experiment (I) and the dry season (November to March) as experiment (II) in 2008-2009. A greenhouse experiment was carried out hydroponically as experiment (III) at the Department of Agronomy, Kasetsart University, Thailand from September to November in 2009.

Experiments (I & II)

Field Experiments

1. Materials

1.1 Plant Materials

Soybean SJ5 cultivar was used in field experiments. This cultivar was developed by crossing between Tainung 4 and SJ2 cultivars released by the Department of Agriculture, Ministry of Agriculture and Co-operative, Thailand in 1980 (Sarobol *et al.*, 2007).

1.2 Equipment

(1) Soil sampling and soil analysis materials
(2) Planter, grinding machine
(3) Electronic balance, oven, leaf punch, moisture meter
(4) Wire tags, plastic and paper bags, nylon mesh bags
(5) Harvesting and yield data collection materials
(6) N and protein content analysis materials and implement

1.3 Chemicals

(1) Urea fertilizer (46% N)
(2) Phosphorous fertilizer (45% P_2O_5)
(3) Potassium fertilizer (60% K_2O)

2. Methods

2.1 Experimental Design

Field experiments were conducted at the National Corn and Sorghum Research Center, Nakhon Ratchasima, Thailand (Latitude: 14º 38′ N, Longitude: 101º 19′ E, Altitude: 387.92 m). Each experiment was arranged in a randomized complete block (RCB) split-plot design with three replications. Four arrangements of plant population density (PPD): 50 cm x 20 cm x 2 plants per hill (20 plants m^{-2}), 50 cm x 20 cm x 3 plants per hill (30 plants m^{-2}), 50 cm x 10 cm x 2 plants per hill (40 plants m^{-2}), 50 cm x 10 cm x 3 plants per hill (60 plants m^{-2}): were assigned to the main plots, and four levels of N starter rate: 0 (control), 25, 50, 75 kg ha^{-1}: were randomly applied within each main plot. Individual plot size was 4 m wide and 5 m long. In each sub-plot eight rows of soybean (cv. SJ5) were planted at different seasons (wet and dry) in 2008-2009.

2.2 Cultural Practices

The experimental plot was prepared for seeding and sown six seeds per hill in each row. Two weeks after sowing, thinning was done to get final populations of 200,000 (20 plants m^{-2}), 300,000 (30 plants m^{-2}), 400,000 (40 plants m^{-2}) and 600,000 (60 plants m^{-2}) plants per hectare. For the basal fertilizer application, 56.25 kg ha^{-1} P_2O_5 as triple super phosphate (45% P_2O_5) and 37.5 kg ha^{-1} K_2O (60% K_2O) (Chainuvati *et al*., 1994) were applied in all sub-plots. Four N treatments were supplied in the form of urea (46% N) as starter doses. Irrigation practices were done due to the farm regulation. Pest and disease management was controlled whenever necessary. The two middle rows of each plot, 4 m long, were harvested for seed yield. To remove any border effects, near border from the plot ends were left when harvest.

2.3 Sampling Procedures

Plants were sampled three times during the growing season; starting at the vegetative stage (V5), at the beginning seed stage (R5), with a final sampling at maturity stage (R8). Growth stages were described according to Fehr and Caviness (1977).

2.3.1 Soil sampling

Just prior to the start of the experiments, soil samples from the experimental site were collected to determine the nutrient content in the soil. After conducting the experiments, nutrient balance of soil from experimental sites was measured in comparison with its initial levels. Soil samples were analyzed at the Soil Science Department, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand. The experimental soil was composed of 19% sand, 12% silt, 69% clay (texture C), and medium amount of organic matter of 1.8% with pH 6.8. The previous crop of the experimental site was corn.

2.3.2 Dry Matter Distribution at V5 and R5

At V5 and R5 stages, 10 randomly selected plants were sampled from each treatment. Plants were partitioned into leaves, stems, roots at V5, and pods were separated into seeds and pod walls at R5. Fresh weight (FW) of all samples was recorded just after harvesting and oven-dried at 65ºC for 72 h. Dry Matter of plant parts was measured. The SLW was also sampled at V5 and R5 growth stages. Ten youngest fully expanded leaflets (YFEL) were harvested from five plants in each plot. Leaf samples were kept in plastic bags and placed on ice immediately until taking FW. Two leaf discs, 10 mm in diameter, were taken from each sampled leaflet with a leaf-punch. Care was taken to avoid major veins, leaf margins, and damages. The punches were stored in paper envelopes, oven-dried at 65ºC for 48 h, and weighed on an electronic balance.

2.3.3 Yield components and seed yield

At the maturity stage (R8), yield components, *viz.* number of branches per plant, number of pods per plant, number of seeds per pod and 100-seed weight, were collected on a randomly selected subsample of 10 plants. Total seed yield was determined from a harvested area of 4 m^2 and it was adjusted to 130 g kg^{-1} moisture.

2.3.4 Protein and oil analysis

For measuring protein and oil concentration in the seed, seeds were ground into a powder that provided maximum homogeneity and minimum sampling variation. Homogenates were determined in whole grain samples by near infrared spectroscopy (NIRS) method. The calibration process was described by Rippke *et al*. (1996) and was subsequently made into a standard method of the American Association of Central Chemistry (1999).

2.4 Parameters of Nitrogen Nutrition in Soybean

The following parameters were calculated to understand the nitrogen nutrition in soybean at V5 and R5-stage.

Specific leaf weight (SLW) and leaf water content (LWC) were calculated using the equations defined by Steinbauer (2001) as follows:

$$SLW\ (mg/cm^2) = \frac{dry\ weight\ of\ leaf\ discs}{area\ of\ hole\ punch}$$

$$LWC\ (\%) = \frac{(leaf\ fresh\ weight - leaf\ dry\ weight)}{leaf\ fresh\ weight} \times 100$$

2.5 Statistical Analysis

All resulted data were subjected to analysis of variance (ANOVA) appropriate for a randomized complete block (RCB) split-plot design. Separate analysis of variance was performed for each measurement. Mean separations were accomplished using Fisher's LSD test at 5% level. Correlations among parameters were analyzed using IRRISAT version 5.0.

Experiment (III)

Hydroponics Experiment

1. Materials

1.1 Plant Materials

Three soybean cultivars were used in this experiment. These are;

(1) Chakkrabbhandhu 1 (CKB1) originated from the cross of UFC1 and Santa Rosa

(2) SJ5 originated from the cross of Tainung 4 and SJ2, and

(3) Chiangmai 60 (CM60) originated from the cross of Williams and SJ4

1.2 Equipment

(1) Germination paper
(2) Plastic string
(3) Bamboos
(4) 75 lit plastic buckets
(5) pH and electrical conductivity (EC) meter
(6) Chlorophyll meter (SPAD-502)
(7) Oven, electronic balance, leaf punch

(8) Grinding machine
(9) Hydroponic apparatus

1.3 Chemicals

1.3.1 Chemicals for seed germination

(1) 0.1 m*M* $CaSO_4$ solution
(2) Deionized water

1.3.2 Chemicals for basic solution (Hoagland solution)

1.3.2.1 Macro nutrients

(1) $Ca(NO_3)_2$
(2) KNO_3
(3) $MgSO_4$
(4) KH_2PO_4

1.3.2.2 Micro nutrients

(1) H_3BO_3
(2) $MnCl_2$
(3) $CuSO_4$
(4) $ZnSO_4$
(5) $NaMoO_4$

1.3.2.3 Fe-EDTA

(1) $FeSO_4.7H_2O$
(2) EDTA

2. Methods

2.1 Experimental Design

The experiment was conducted in a randomized complete block (RCB) split-plot design with three replications. The main plots consisted of three levels of nitrate-nitrogen (5 m*M*, 10 m*M*, 15 m*M*). The concentration of NO_3^- in the three treatments were: 1) 5 m*M* NO_3^- basal solution used as a control (1.5 m*M* $Ca(NO_3)_2$ + 2 m*M* KNO_3); 2) 10 m*M* NO_3^- (3.5 m*M* $Ca(NO_3)_2$ + 3 m*M* KNO_3); and 3) 15 m*M* NO_3^- (5.5 m*M* $Ca(NO_3)_2$ + 4 m*M* KNO_3). Three soybean cultivars (CKB1, SJ5, CM60) were arranged in the sub-plots.

2.2 Cultural Practices

2.2.1 Plant culture

Seeds of three soybean cultivars; Chakkrabbhandhu 1 (CKB1), SJ5 and Chiangmai 60 (CM60), were pre-germinated in germination paper at normal room temperature and RH in the lab. The germinating seeds were kept moist by capillary action from a 0.1 m*M* $CaSO_4$ solution.

2.2.2 Nutritional Treatments

Initial concentrations of nutrients in the basic solution (Hoagland solution) were macronutrients: $Ca(NO_3)_2$ 1.5 m*M*, KNO_3 2 m*M*, $MgSO_4$ 1 m*M*, KH_2PO_4 1 m*M*; micronutrients: H_3BO_3 46.2 µ*M*, $MnCl_2$ 9.2 µ*M*, $CuSO_4$ 0.38 µ*M*, $ZnSO_4$ 2.4 µ*M*, $NaMoO_4$ 1.2µ*M*; and Fe-EDTA: $FeSO_4.7H_2O$ 90 m*M*, EDTA 90 m*M*. All solutions were made up of deionized water. The basic nutrient solution pH was ranged from 5.5 to 7.0 and EC was about 1.66 mS cm^{-1}. Plants were supplied only basic solutions from the day of transplanting until 4 DAT. The concentration of NO_3^- in the basic solution was considered as a control (5 m*M*). After 4 DAT the two levels of NO_3^- treatments were started by adding KNO_3 and $Ca(NO_3)_2$ solutions to establish required concentrations of 10 m*M* and 15 m*M* proportionally. The initial pH of all nutrient treatments was adjusted to maintain with 1N HCl.

2.2.3 Handling of plants

After 4 d, 81 vigorous uniform-sized seedlings were selected and transplanted into nine 25-L recipients covered with Styrofoam tops. Each seedling was suspended in a container supported with coarse perlite. The 25-L recipients were separated into three blocks according to NO_3^- levels. Each block consisted of three 25-L pipes connected to the under table compartment, 75-L plastic bucket, provided with a pump to circulate the nutrient solution to continuous flow. Fifteen days after transplanting (DAT), plants were supported against the strings running between the poles to grow upward and to obtain enough light until final harvest.

2.2.4 Handling of nutrient solutions

Adjustment of nutrient levels of the under table buckets, and measurement of solution pH and EC were monitored daily. To avoid nutrient depletion effects, the nutrient solution was replenished 18 DAT and 30 DAT. At this time, the solution in each bucket was sampled to determine pH and EC, and then circulation between the two compartments was discontinued. After emptied the solutions in the upper recipients into the under table buckets the entire solutions in the under table buckets were drained. Fresh nutrient solutions were refilled in the under table buckets, and then the circulation system was continued. This process takes less than 10 min to allow for replenishment of fresh nutrient solution into the hydroponic system with minimal disturbance to plant function. Throughout the growing season, plants were exposed to natural light intensity, temperature, humidity and photoperiod in the greenhouse.

2.3 Sampling Procedures

Three replicates of the control and NO_3^--treated plants of three soybean cultivars were harvested at V5, R5 and R8 (maturity). The roots of plant samples were rinsed in tap water thoroughly, blotted dry in paper towels and plants were separated into leaves with petioles, stems, roots at V5 and pods were also divided into pod walls and seeds at R5. Every plant part was oven-dried at 65°C for 72 h. Dry mass of plant parts and whole plants were measured. Specific leaf weight was determined at V5 and R5 stages on the basic of fresh- and dry- weight. At final harvest (R8), number of branches per main stem, number of pods per plant, dry weight of stems, roots, pod walls and seeds per plant were recorded.

2.4. Shoot/root ratio

Biomass partitioning was determined between aboveground (leaves, stems, flowers, fruits) and belowground (roots) (shoot: root). This was performed using the classical method of growth analysis by Blackman (1919).

$$Shoot/Root\,ratio = {}^{S}/_{R}$$

Where S = weight of shoot (above cotyledonary node)

R = weight of root (below cotyledonary node)

2.5 Chlorophyll Content

Chlorophyll content was measured with a photometer, SPAD-502 chlorophyll meter (Spectrum Technologies, Inc.). The SPAD readings are calibrated to obtain the chlorophyll content of the leaves (Peltonen *et al.*, 1995), providing a practical method of assessing N status and N requirements.

2.6 Tissue N and C Analysis

For total N and C analysis, subsamples of leaves, pod walls and seeds at R5 and pod walls and seeds at R8 were pulverized to a fineness that provided maximum homogeneity and minimum sampling variation. The nitrogen (N), hydrogen (H) and carbon (C) contents were determined with Elementary CHN Autoanalyzer (TruSpec CHN, LECO. Corp., St. Joseph, Michigan, USA) in the Central Lab at Faculty of Agriculture, Kasetsart University, according to ASTM D5373-93 (2002). Nitrogen percentage in seeds was converted to protein percentage by multiplying the convertion factor, 6.25.

2.7 Statistical Analysis

Data were subjected to analysis of variance (ANOVA) appropriate for a randomized complete block (RCB) split-plot design. Separate analysis of variance was performed for each measurement. Simple correlation coefficient and regression analysis for different parameters were computed. Mean separations were accomplished using LSD test at 5% level.

RESULTS AND DISCUSSIONS

Field Experiments (Experiment I & II)

1. Plant Growth Analysis

Since growth parameter analysis provides a framework for identifying potentially useful traits for yield improvement and possibly the physiological explanation for the yield response (Ball *et al.*, 2001), growth response of soybean SJ5 cultivar to N fertilization was observed at the four arrangements of PPD in two different seasons. The stage of development was assessed for all cultivars in the field and greenhouse experiments by the method of Fehr and Caviness (1977).

1.1 Specific Leaf Weight (SLW) and Leaf Water Content (LWC)

In the wet season, SLW was gradually increased with increasing PPD from 20 to 40 plants m^{-2} at V5 stage and from 20 to 30 plants m^{-2} at R5 stage, respectively (Figure 1.A). The lowest SLW values at both growth stages were found at the maximum PPD (60 plants m^{-2}). On the other hand, N fertilizer as a starter significantly increased SLW at both V5 and R5 stages (Figure 1.B). There was a steady increase in SLW-R5, but was no linear progress at V5. However, SLW-V5 was rapidly increased when N fertilizer supplied from 0 to 25 kg ha^{-1}. No more increase in SLW was observed when N fertilizer rate was added from 25 to 50 and thereafter it increased from 50 to75 kg ha^{-1}. The highest N rate (75 kg ha^{-1}) resulted the largest SLW at both V5 and R5 stages. Compared to control, the highest N rate produced a greater increase in SLW by 43.8 % and 17.4% at V5 and R5, respectively.

In the dry season, PPD and N fertilization showed significant effects on SLW at R5, but not at V5 (Figure 2.A and B). At both growth stages, SLW was slightly lower by increasing PPD and the lowest value was found at the highest PPD (60 plants m^{-2}). In general, SLW was reduced by higher PPD with narrow row spacing. While the greatest SLW was measured at the highest N level (75 kg ha^{-1}) at both growth stages, N treatment did not make statistical impact on SLW at V5 (Figure 2.B). SLW value in dry season was larger at R5 stage but lower at V5 stage than in wet season (Figure 1.B and 2.B). The results indicate that SLW of soybean response to N starter application is mainly associated with developmental stage and growing season. In contrast, Peng *et al*. (1993) evaluated the effect of N fertilizer on the SLW in rice, and they suggested that N fertilization in rice was minor and inconsistent in comparison with the effects of growth stage. Similarly, lack effect of N fertilizer on the SLW in sunflower was demonstrated by Cechin and Fumis (2004).

In the present study, the largest SLW values (5 mg cm^{-2}) at R5 stage resulted by the lowest PPD and the highest N rate were observed in the dry season (Figure 2). In addition, the maximum temperature at R5 stage (27.3 ^{o}C) in the dry season was lower than that (31.7 ^{o}C) in the wet season (see Appendices C and D). According to Lugg and Sinclair (1980), leaf thickening was largely the result of concurrent thickening of palisade and spongy mesophyll tissues and the final SLW and leaf thickness obtained were modified by solar radiation levels during the period of leaf development. Chabot (1977) stated that thick leaves with low density mesophyll became thinner and denser with increasing growth temperature corresponding to an increase in maximum net photosynthetic rates. Leaves became thicker and denser at the highest temperatures, but with an increase in cell damage and indications of changes in metabolic pathways. Hence, the SLW on dry basis increased under reduced temperature.

Effects of N starter rate and PPD on LWC of soybean SJ5 cultivar at V5 and R5 stages grown in the fields are shown in Figures 3 and 4. In the wet season, PPD significantly affected LWC at V5 and R5 ($p = 0.01$) (Figure 3.A). The lowest LWC was observed from PPD of 30 plants m^{-2} at R5 stage. Nitrogen application had significant influences upon LWC at V5 ($p< 0.01$) and at R5 ($p = 0.01$) (Figure 3.B). Leaf water content was decreased and increased with increased N fertilizer application at V5 and R5 stage, respectively. At V5 stage, LWC was gradually reduced by increasing N application from control (0 kg ha^{-1}) to the highest rate (75 kg ha^{-1}). Conversely, at R5 stage, LWC began with a minimum value at control N rate and smoothly increase till the highest N rate. In the dry season, there were significant differences in LWC among PPD at R5 ($p< 0.05$) (Figure 4.A). The lowest LWC was produced by the highest PPD (60 plants m^{-2}) at R5 stage. In this season, starter N application had negative effects on LWC at both stages (Figure 4.B). Therefore, the lowest LWC was observed at the highest N rate.

Data on N fertilizer influencing SLW and LWC showed that the expression of SLW and LWC are dependent upon growth stages and growing seasons (Figures 1, 2, 3 and 4). At V5, wet season growing plants had 0.71 mg cm^{-2} greater in SLW and 4.88% lower in LWC than dry season growing plants. At R5, wet season plants had 0.4 mg cm^{-2} and 3.02% lower in SLW and in LWC, respectively, than dry season growing plants. In the case of leaf age, Steinbauer (2001) found that old leaves had greater SLW (± 0.03 mg/mm^2) than new leaves and were (± 6%) lower in LWC than new leaves of Tasmanian bluegum (*Eucalyptus globulus* ssp. *globules*).

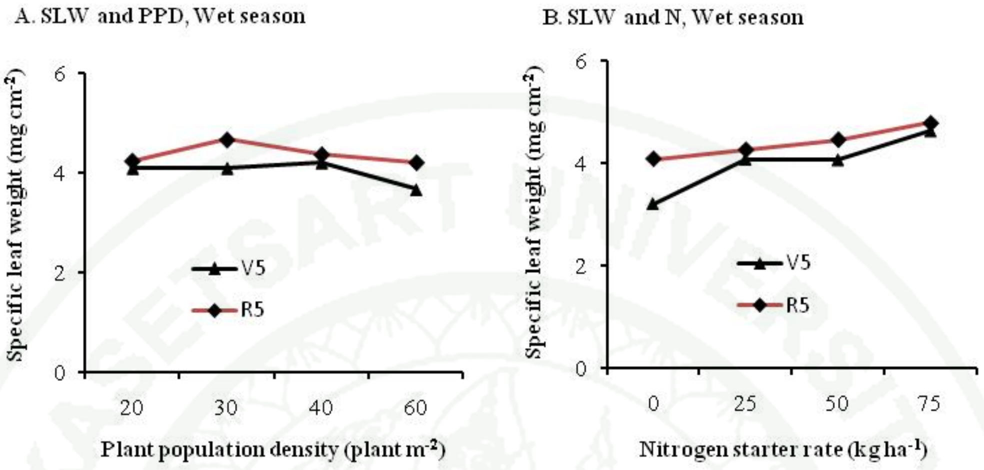

Figure 1 Effects of plant population density (PPD) (A) and N starter rate (B) on specific leaf dry weight (SLW) of soybean SJ5 cultivar at V5 and R5 stages in the wet season.

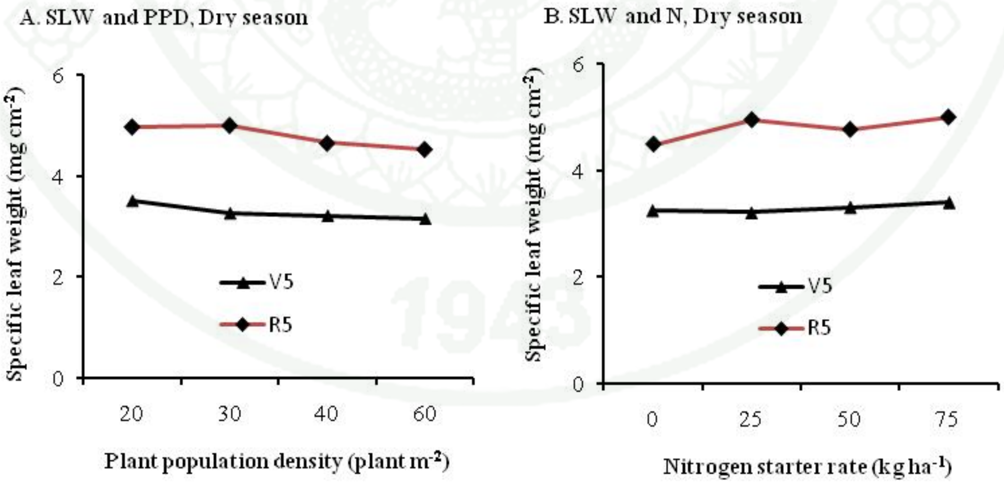

Figure 2 Effects of plant population density (PPD) (A) and N starter rate (B) on specific leaf dry weight (SLW) of soybean SJ5 cultivar at V5 and R5 stages in the dry season.

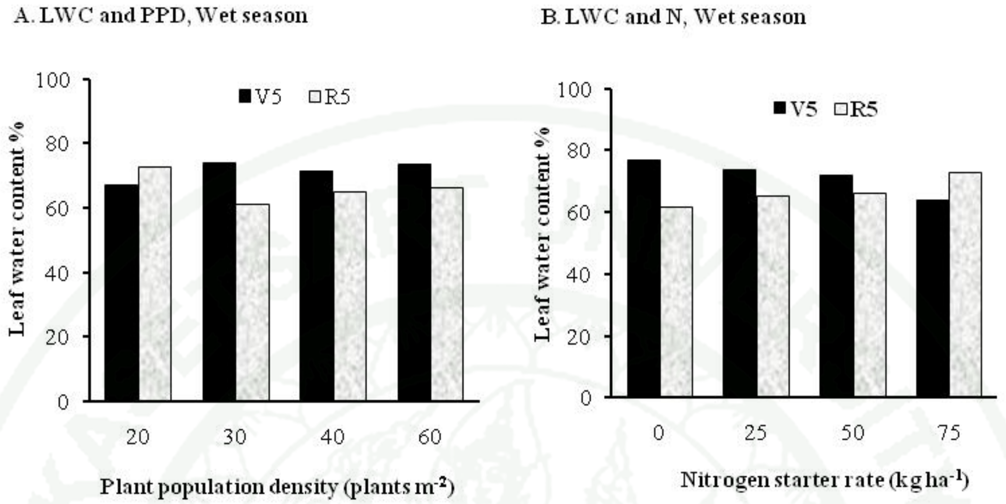

Figure 3 Effects of plant population density (PPD) (A) and N starter rate (B) on leaf water content (LWC) of soybean SJ5 cultivar at V5 and R5 stages in the wet season.

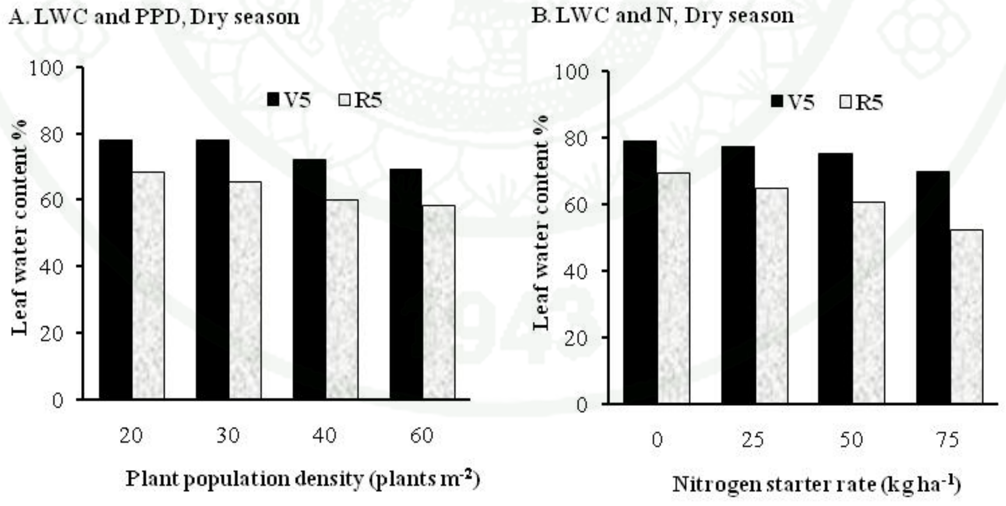

Figure 4 Effects of plant population density (PPD) (A) and N starter rate (B) on leaf water content (LWC) of soybean SJ5 cultivar at V5 and R5 stages in the dry season.

1.2 Dry Matter (DM) Distribution

1.2.1 Vegetative Stage (V5)

Effects of N starter rate and PPD on DM distribution in soybean SJ5 cultivar were observed in the wet and the dry seasons. There were no treatment interactions in all observations (Table 1).

In the wet season, DM accumulation in plant parts was affected by PPD. Increase in TDM by an average of 77% was recorded when PPD was shifted from 20 to 30 plants m^{-2}. Dry matter of all plant parts was increased with increasing PPD from 20 until 60 plants m^{-2}. Therefore maximum TDM (kg ha^{-1}) was produced by the highest PPD of 60 plants m^{-2} due to earlier canopy closure. Thus, the data revealed that total crop dry matter is the spatial and temporal integration of all plant processes and, crop canopy is the most relevant parameter of crop dry matter. Nitrogen application had a positive impact on DM of all plant parts at V5. Total dry matter yield was increased by an average of 23% when N rate was increased from 0 to 75 kg ha^{-1}. Maximum TDM receiving the highest N rate of 75 kg ha^{-1} was mainly associated with the largest value of DM accumulation in the leaves. On average, 62% of total biomass was accumulated in leaves. Root DM of plants receiving 75 kg ha^{-1} showed similar value compared with that of plants receiving 25 and 50 kg ha^{-1}. Similar results have been reported by (Rabie *et al*., 1980, Finke *et al*., 1982 and Harper, 1987) that under sustained supply of N, crop produced greater quantity of biomass and shared its greater proportion to stems compared with roots.

In the dry season, PPD positively affected TDM (kg ha^{-1}) of soybean SJ5 cultivar at V5 stage. The maximum DM in the whole plant (687.2 kg ha^{-1}) was observed at the highest PPD (60 plants m^{-2}). Compared to the lowest PPD (20 plants m^{-2}), the highest PPD gave 51% higher in TDM. Application of N starter fertilizer made no significant impact on DM of any plant part at V5. Although there were no significant differences in DM accumulation due to N fertilization in dry season, it was noted that the highest N rate of 75 kg ha^{-1} possessed the slightly higher in TDM than the other rates. On average, 65% of total biomass accumulated in the leaves. Regardless of the treatments, the wet season growing plants increased in TDM twice as compared with the dry season growing plants. This increase in TDM was due primarily to the plant height (data not shown). During growing period, plants received greater amount of rainfall in the wet season than that in the dry season (see Appandices C and D), resulting excessive plant growth in the wet season.

1.2.2 Reproductive Stage (R5)

Responses of DM accumulation in each plant part and TDM influenced by N starter rates and PPD were observed at R5 stage in two different seasons. In both seasons, TDM yields (kg ha^{-1}) were increased with increasing N fertilizer application and PPD (Table 2).

In the wet season, there were significant differences in DM of vegetative plant parts (i.e. leaf, stem and root), but was no difference in DM of reproductive plant part (i.e. pod) due to PPD. All of the vegetative plant parts resulted by the highest PPD (60 plants m^{-2}) showed the highest DM in each vegetative plant part. Therefore, the maximum TDM per unit area was observed at the highest PPD. Total biomass yield (12204.6 kg ha^{-1}) produced by the highest PPD was 93% higher than did by the lowest PPD. Application of N fertilizer had a significant impact on the whole plant biomass. Nitrogen treated plants gave the higher DM than control plants. Relative to control (0 kg ha^{-1}), N rates of 25 and 75 kg ha^{-1} increased whole plant biomass by an average of 14%. This increase was mainly associated with leaf DM for both N rates. On average, DM accumulation in leaves accounted for 58% of total biomass. Increased total DM yields due to supplemental N have also been reported by Lawn and Brun (1974) and Eaglesham *et al.* (1983). The greater increase in leaf DM in this season implies that application of the N fertilizer as a starter stimulated vegetative development more than reproductive growth.

In the dry season, accumulation of DM in all plant parts as well as TDM were significantly affected by PPD at R5 (Table 2). There was linearly increase in TDM when PPD was increased from 30 to 60 plants m^{-2}. This increase in TDM resulted by the higher PPDs could be due to the substantial amount of vegetative DM accumulation in leaves and stems. The highest PPD produced the greatest TDM yield (3897.4 kg ha^{-1}) by an average of 88% higher than did the lowest PPD. Ball *et al.* (2000) also reported that the highest population level ensured early canopy coverage and maximized light interception, greater crop growth rate and crop biomass. In this season N fertilization significantly impacted on DM accumulation in every plant part at R5 stage. Increasing the rate of N fertilizer from 0 to 75 resulted in a higher accumulation of pod dry matter by an average of 92%. The largest significant amount of whole plant biomass (3412 kg ha^{-1}) was achieved when the highest amount of N fertilizer (75 kg ha^{-1}) was supplied. At the highest N application, 40% of total plant biomass was accumulated in the leaves.

Results obtained from the two season experiments exhibited that PPD had significant differences in DM accumulation of soybean at vegetative and reproductive stages in both seasons. Increasing PPD positively affected DM in all observations. Likewise, starter N application increased the TDM (i.e. whole plant DM) yield in soybean in both seasons. This may lead to the higher seed yield because grain yield is positively correlated with vegetative biomass (Frederick *et al*., 1991). In addition to the higher seed yields (Table 4), TDM yields were increased over-all by average of 14 % in the wet season and 53% in the dry season when N fertilizer rate was increased from control (0 kg ha^{-1}) to the highest level (75 kg ha^{-1}). This result agreed with the reviewed data of Salvagiotti *et al*. (2008) who stated that high yielding soybean requires large amounts of N to support the above-ground biomass.

According to Shibles and Sundberg, (1998), high yields of soybean are associated with leaf mass at R5 (the beginning of seed growth) and DM-R5 was more than twice as important as leaf N concentration in establishing the amount of mobilizable N. In accordance with their results, growth response of soybean SJ5 cultivar grown with N fertilization showed that DM was largely accumulated in leaves, indicating the major factor for high seed yield in this study. In soybeans, there was a linear relationship between increase in plant DM increase and seed DM; half of the dry matter produced during the SFP was consumed for vegetative organs and the remaining half for seed production in diverse soybean cultivars, and the reserves stored before flowering apparently did not contribute to seed production (Kakiuchi and Kobata, 2004). The present research suggests that TDM accumulation during their formation period would play an important role in yield formation.

Differential responses of DM partitioning in reproductive plant parts at R5 stage to N fertilization and PPD under field conditions were separately analyzed (Table 3). It was apparent that seed DM did not respond to varying N rate and PPD in the wet season. Conversely, the significant variation in the seed DM was occurred due to increasing N rate and PPD in the dry season. The nutrient treatment had a considerable effect on the pod wall DM in both seasons. In both seasons, the lowest rate of N application (0 kg ha^{-1}) had a minimum pod wall DM yield. No significant difference in pod wall DM was found by increasing PPD in each season. Although total pod DM was not altered by application of N fertilizer in the wet season (Table 2), DM accumulation in pod wall was influenced by N fertilization (Table 3). This finding supports the suggestion of Van der Werf and Nagel (1996) that N fertilization not only leads to overall increased growth and biomass production, but also results in alterations in the allocation of resources and in plant morphology.

Table 1 Effects of nitrogen rate and plant population density on dry matter in plant parts and total dry matter (TDM) of soybean SJ5 cultivar at V5 stage in two different seasons.

PPD (plants m^{-2})	N rate (kg ha^{-1})	Leaf	Stem	Root	TDM
		(kg ha^{-1})			
			Wet Season		
20 plants m^{-2}		285.3 c[§]	91.3 d	86.8 c	463.4 d
30 plants m^{-2}		508.6 b	157.5 c	153.2 b	819.4 c
40 plants m^{-2}		585.6 b	193.6 b	162.0 b	941.2 b
60 plants m^{-2}		903.8 a	308.3 a	270.6 a	1482.6 a
LSD $_{0.05}$		51.7**	23.8**	12.2**	67.0**
	0	502.0 c	175.5	150.1 b	827.6 c
	25	571.4 b	184.3	171.7 a	927.4 b
	50	575.1 b	185.4	170.0 a	930.4 b
	75	634.7 a	205.6	180.9 a	1021.2 a
LSD $_{0.05}$		51.7**	NS	12.2**	67.0**
CV(a) %		26	20	16	22
CV(b) %		11	15	9	9
			Dry Season		
20 plants m^{-2}		228.3 d	63.2 d	48.2 c	339.6 d
30 plants m^{-2}		282.6 c	86.3 c	58.2 c	427.1 c
40 plants m^{-2}		366.5 b	114.3 b	85.3 b	566.1 b
60 plants m^{-2}		420.6 a	154.0 a	112.7 a	687.2 a
LSD $_{0.05}$		31.7**	11.1**	11.8**	43.3**
	0	308.0	101.5	77.7	487.1
	25	315.5	101.1	74.1	490.7
	50	334.3	109.6	73.4	517.3
	75	340.3	105.5	79.2	525.0
LSD $_{0.05}$		NS	NS	NS	NS
CV(a) %		29	27.0	25	26
CV(b) %		11	13.0	18	10

§ Within column, means followed by the same letter are not significantly different at the 0.05 probability level by Fisher's LSD.

*,** Significantly different at the 0.05 and 0.01 probability levels, respectively.

NS = non-significant at the 0.05 probability level.

Table 2 Effects of nitrogen rate and plant population density on dry matter in plant parts and total dry matter (TDM) of soybean SJ5 cultivar at R5 stage in two different seasons.

PPD	N rate	Leaf	Stem	Root	Pod	TDM
(plants m^{-2})	(kg ha^{-1})			(kg ha^{-1})		
				Wet season		
20 plants m^{-2}		3575.0 c[§]	1848.3 c	626.7 d	275.9	6325.9 c
30 plants m^{-2}		5022.5 b	2567.5 b	875.0 c	354.8	8819.8 b
40 plants m^{-2}		5490.0 b	2823.3 b	960.0 b	378.3	9651.7 b
60 plants m^{-2}		7175.0 a	3371.7 a	1215.0 a	442.9	12204.6 a
LSD $_{0.05}$		621.2**	385.7**	122.5**	NS	989.7**
	0	4929.2 b	2573.3	918.3	260.1	8680.9 b
	25	5433.3 a	2720.8	923.3	386.2	9463.7 a
	50	5144.2 b	2516.7	873.3	389.8	8924.0 b
	75	5755.8 a	2800.0	961.7	415.9	9933.4 a
LSD $_{0.05}$		621.2*	NS	NS	NS	989.7*
CV(a) %		14	25	10	24	12
CV(b) %		14	17	15	23	13
				Dry season		
20 plants m^{-2}		824.0 b	819.0 c	300.8 c	126.1 c	2069.9 c
30 plants m^{-2}		951.1 b	741.3 c	327.1 c	176.6 c	2196.1 c
40 plants m^{-2}		1187.3 a	1045.6 a	434.8 b	264.9 b	2932.5 b
60 plants m^{-2}		1306.8 a	1584.0 a	622.1 a	384.5 a	3897.4 a
LSD $_{0.05}$		260.2**	121.9**	33.2**	59.4**	359.1**
	0	853.4 b	847.2 c	375.7 c	157.6 c	2233.9 c
	25	914.0 b	999.2 b	423.8 b	253.3 c	2590.3 bc
	50	1121.1 a	1083.2 b	417.3 b	238.2 b	2859.8 b
	75	1380.6 a	1260.5 a	467.9 a	303.0 a	3412.0 a
LSD $_{0.05}$		260.2**	121.9**	33.2**	59.4**	359.1**
CV(a) %		25	27	15	22	20
CV(b) %		14	13	9	14	12

§ Within column, means followed by the same letter are not significantly different at the 0.05 probability level by Fisher's LSD.

*,** Significantly different at the 0.05 and 0.01 probability levels, respectively.

NS = non-significant at the 0.05 probability level.

Table 3 Effects of nitrogen rate and plant population density on dry matter in seed and pod wall of soybean SJ5 cultivar at R5 stage in two different seasons.

PPD (plants m^{-2})	N rate (kg ha^{-1})	Wet season		Dry season	
		Seed	Pod wall	Seed	Pod wall
		(g plant^{-1})			
20 plants m^{-2}		1.24	10.48	0.38 bc[§]	5.92
30 plants m^{-2}		1.42	8.40	0.33 c	5.56
40 plants m^{-2}		0.91	7.25	0.53 a	6.10
60 plants m^{-2}		0.87	7.03	0.33 c	6.08
LSD $_{0.05}$		NS	NS	0.14*	NS
	0	0.95	5.25 b	0.30 b	3.86 c
	25	1.21	9.12 a	0.50 a	6.03 b
	50	0.93	9.02 a	0.34 b	6.30 a
	75	1.36	9.78 a	0.43 ab	7.46 a
LSD $_{0.05}$		NS	3.25*	0.14*	1.38**
CV(a) %		21.4	14.7	28.0	21.5
CV(b) %		31.2	15.5	20.5	14.0

§ Within column, means followed by the same letter are not significantly different at the 0.05 probability level by Fisher's LSD.
*,** Significantly different at the 0.05 and 0.01 probability levels, respectively.
NS = non-significant at the 0.05 probability level.

1.3 Relationship between organ DM and TDM at R5

Organ dry matter versus TDM over four N treatments was determined to assess the relationship between these parameters. In both seasons, the least-squares linear regression analysis showed that the DM of different plant organs increased linerarly with the increasing level of the total plant DM (Figures 5 and 6).

In the wet season, there were highly significant relationships between leaf DM ($R^2 = 0.93$) (Figure 5.A), stem DM ($R^2 = 0.88$) (Figure 5.B) and TDM (g plant^{-1}). The rank of order of increasing biomass on regression line basis was leaf > stem > root > pod (Figure 5.A, B, C, D). Simillary, DM accumulation in leaf and stem was strongly related with TDM in the dry season (Figure 6.D). The rank of order of increasing biomass on regression line was the same trend as in the wet season. Reflecting this trend, the relationship of organ DM with TDM was higher in the wet season than in the dry season. It was noted that, at the beginning seed filling stage (R5), DM accumulation in reproductive organ was weakly linked with TDM. In addition, among the vegetative organs, root showed less relationship with TDM in both seasons. According to the results from field experiments, it is consistent that the amount of total biomass (g plant^{-1}) increased by the effect of starter N and PPD could be derived from DM accumulation in leaf and stem.

2. Yield components and seed Yield

2.1 Seed yield response to PPD

Table 4 summarizes the responses of seed yield and yield components of soybean SJ5 cultivar to varying PPD in two different seasons.

In the wet season, PPD had no significant effect on seed yield per unit area (kg ha^{-1}). Among yield components, the number of branch and pod per plant was significantly affected by PPD. It is notable that yield components such as the number of branch and pod per plant was not changed by increasing PPD from 20 to 30 plants m^{-2}. However, increasing PPD from 30 to 40 plants m^{-2} reduced the number of these two variables. This could be primarily due to narrow plant spacing. In this study, the arrangement of PPD to get 20 and 30 plants m^{-2} was two and three plants hill^{-1}, respectively, in 20 cm intra-row spacing, whereas the higher PPDs of 40 and 60 plants m^{-2} were two and three plants hill^{-1}, respectively, in 10 cm intra-row spacing. Therefore, higher PPD combined with narrow row spacing had a negative effect on the branch and pod production of individual plants. The pod number per plant was decreased with increasing PPD by an average of 37%. This concurred with other studies (Herbert and Litchfield, 1982; Mathew *et al*., 2000) that pod number per plant was the yield component most influenced by changes in cultural and environmental conditions. Liu *et al*. (2010) confirmed pod number per plant was reduced with increasing density for all soybean cultivars grown in 2007.

In the dry season, PPD had a significant effect on seed yield per unit area ($p < 0.05$). Seed yield per unit area increased with increased PPD from 20 to 30 plants m^{-2}, and no more increase in seed yield was recorded when PPD was increased from 30 until 60 plants m^{-2}. Yield components such as 100-seed weight, the number of branch and pod per plant had significant responses to PPD. However, PPD had no effect on seed number per pod not only in the wet season but in the dry season. Similar findings of no significant changes in seed number per pod have been reported in previous studies (Herbert and Litchfield, 1982; Liu, 2010). Although the lowest PPD (20 plants m^{-2}) produced the maximum number of branch per plant and pod per plant, it did not lead to the highest seed yield per unit area. Seed yield resulted from 20 plants m^{-2} was 11, 15 and 9% lower than those resulted from 30, 40 and 60 plants m^{-2}, respectively. The highest PPD (60 plants m^{-2}) possessed a minimum 100-seed weight (14.92 g). Whereas the highest PPD significantly reduced 100 seed-weight, number of branch and pod per plant, it did not decrease seed yield per unit area. It was noted that the reduction in agronomic characters influencing seed yield per plant was compensated by the increasing PPD for seed yield per unit area. This suggests high populations provide a way to optimize grain yields in short-season production systems (Liu *et al*., 2007).

Studies on seed yield response to PPD indicated that adjusting planting density is an important tool to optimize crop growth and the time required for canopy closure, and to achieve maximum biomass and grain yield. Thus, manipulation of planting pattern and density of soybean has resulted in variable growth and seed yield responses (Duncan, 1986; Robinson and Wilcox, 1998; Edwards and Purcell, 2005). Data collected from the field studies illustrated that soybean seed yield response to PPD was influenced by planting arrangement and growing season. In the wet season, the unchanged seed yield (kg ha^{-1}) with increased PPD indicating the lowest PPD (20 plants m^{-2}) would be suitable for tested soybean cultivar (SJ5). In the dry season, whereas significant seed yield was increased by PPDs of 30, 40, and 60 plants m^{-2}, this increment was statistically the same value among these treatments. Thus, 30 plants m^{-2} could be defined as an appropriate PPD for maximizing seed yield in the dry season, indicating the optimum plant population is the minimum population required by the crop to produce maximum yield.

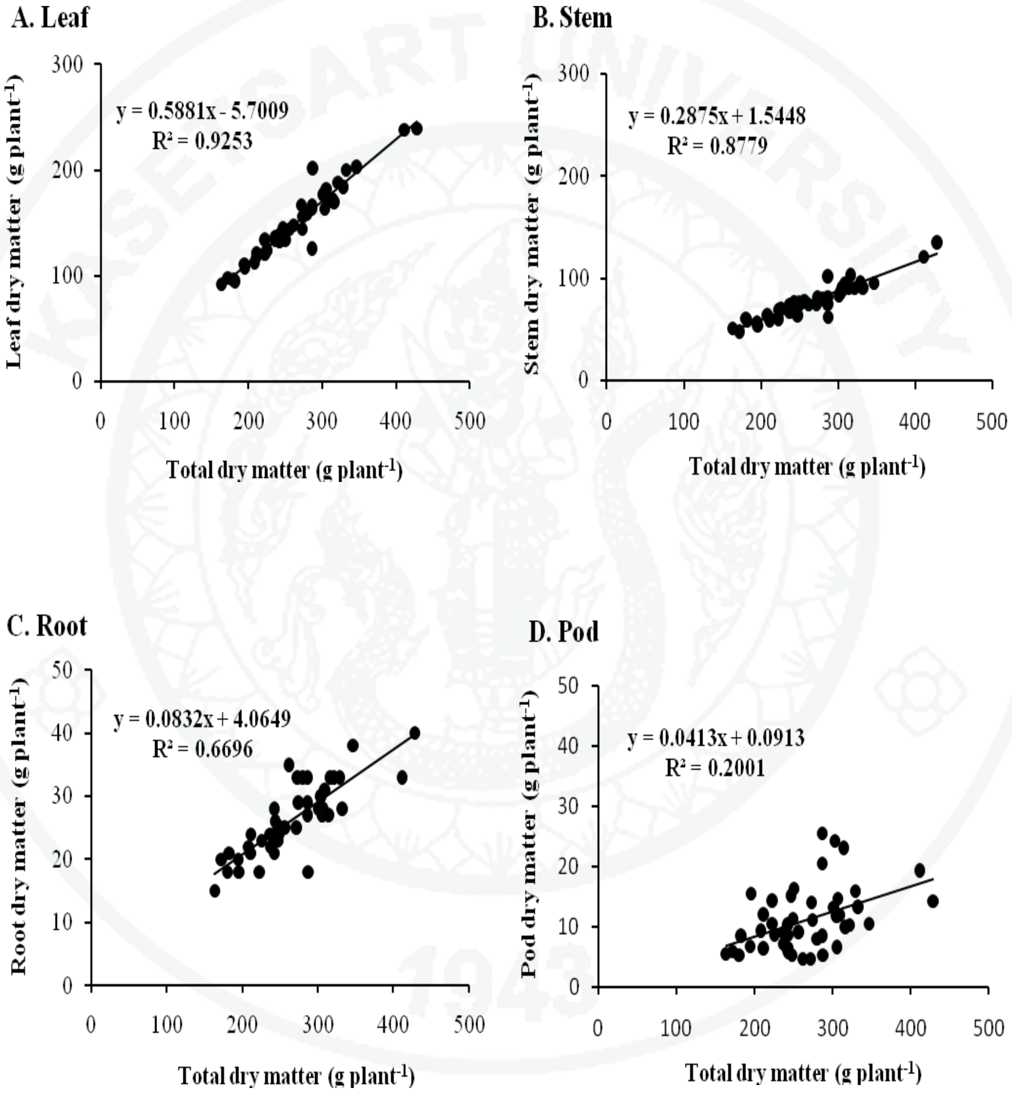

Figure 5 The relationship between organ dry matter and total plant biomass of soybean SJ5 cultivar at R5 stage in the wet season.

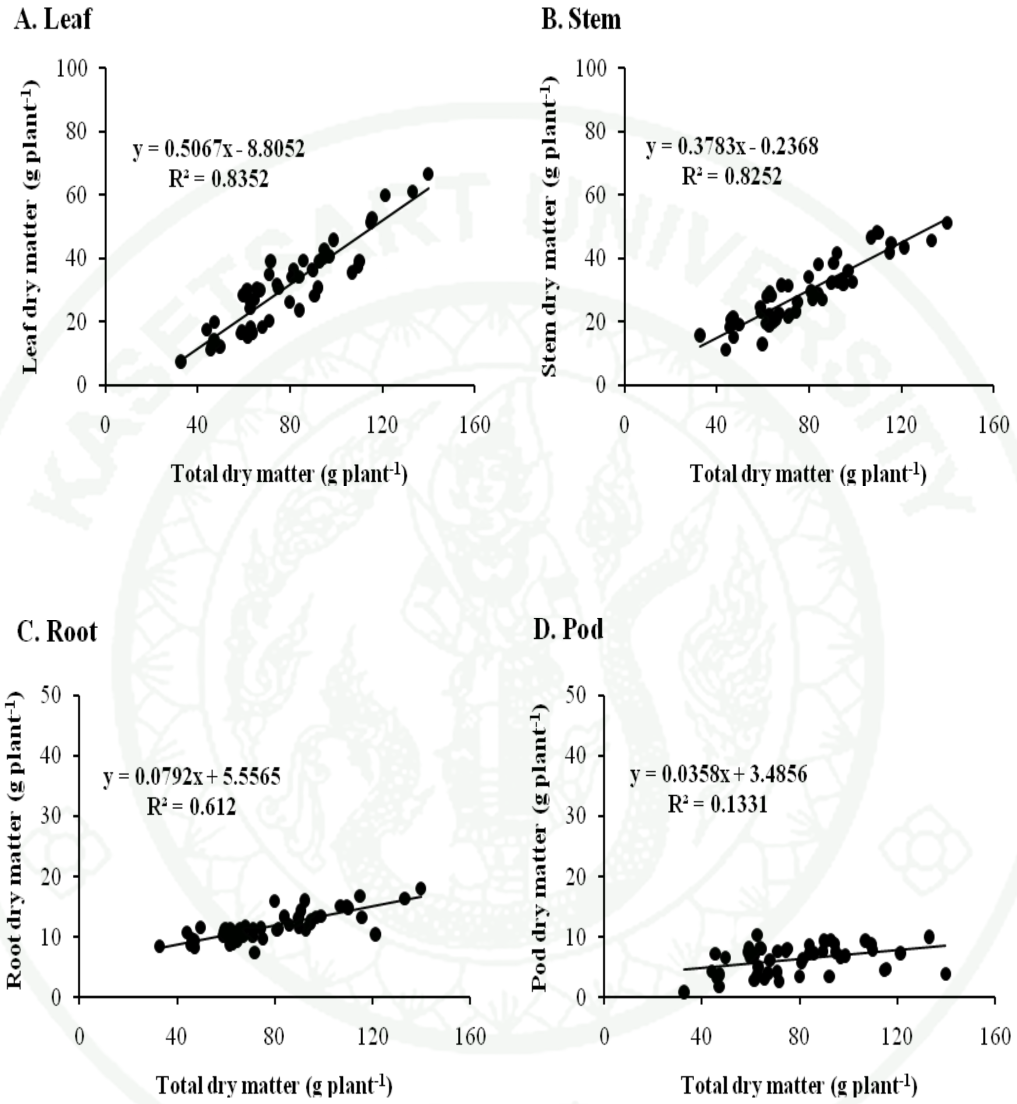

Figure 6 The relationship between organ dry matter and total plant biomass of soybean SJ5 cultivar at R5 stage in the dry season.

2.2 Seed yield response to N fertilization

Starter N fertilizer application had a considerable effect on seed yield per unit area ($p< 0.01$) of soybean SJ5 cultivar grown in two different seasons (Table 5).

In the wet season, N fertilization had a significant difference in seed yield per unit area. Analysis of variance indicates a linear increase in seed yield (kg ha^{-1}) with N application. The response of seed yield ranging from 2174.28 to 2843.06 kg ha^{-1} to varying N rate was observed. The maximum seed yield (2843.06 kg ha^{-1}) was recorded at the highest N rate (75 kg ha^{-1}) and minimum seed yield (2174.28 kg ha^{-1}) was at the control rate (0 kg ha^{-1}). Among yield components, 100-seed weight was highly affected by N fertilization. Relative to control, the highest N rate increased 100-seed weight and seed yield by an average of 10 and 31%, respectively. It seems that the maximum seed yield response to the highest N rate is mainly associated with the maximum seed size (based on 100-seed weight). This result was supported by Sorensen and Penas (1978), who investigated in Nebraska that yield increases were associated with more favorable yield environments as well as increased seed size.

In the dry season, starter N application had significant effects on yield components and seed yield per unit area. It was clearly showed that seed yield was increased from 1017.43 to 1602.52 kg ha^{-1} with increasing N rate from control (0 kg ha^{-1}) to 75 kg ha^{-1}. The highest N rate had a positive impact on pod number per plant, seed number per pod and 100-seed weight. The number of branch per plant was not statistically different from control plants. In addition, pod number per plant and seed number per pod was statistically the same value as compared to 50 kg ha^{-1} N treatment. Hence the greatest seed yield (1,602.52 kg ha^{-1}) resulted by the highest N rate was mainly associated with higher value of 100-seed weight. This suggests that the application of N fertilizer to soybean increased the seed yield which was associated with increase in 100-seed weight. On average, the number of pod per plant, seed per pod, 100-seed weight, and seed yield obtained from 75 kg ha^{-1} treatment were 40, 7, 8 and 57%, respectively, superior to control.

Since agronomic efficiency of applied N varied widely due to large variation in indigenous soil N supply, N rates, application methods, and other factors affecting yield responses to N application (Salvagiotti *et al*., 2008), several researchers detected the inconsistent responses of applied N with various levels of treatments under different environments. Osborne and Riedell (2006) observed increased soybean seed yield by application of starter N in two out of three years. Barker and Sawyer (2005) evaluated the effect of in-season (R3) N application to soybean in Iowa and showed that soybean grain yield was not affected by fertilizer N application. However, plant N concentration and plant DM were increased. Research in Virginia on irrigated soybean revealed a consistent lack of response to N applications during reproductive stages (Freeborn *et al*., 2001). Beard and Hoover (1971) also concluded that no benefit to N application during the R2 stage on irrigated soybean. Conversely, this study exhibited consistent effects of N fertilizer application as a starter on soybean seed yield under field conditions. In both seasons, a positive effect of N fertilizer on seed yield per unit area was observed in the range of 0 to 75 kg ha^{-1}. Regardless of the treatments, greater seed yield in the wet season than in the dry season seems likely to be mainly associated with higher TDM in the wet season (Table 2 and 3). It was also assumption that the higher relative humidity (RH) during the flowering phase (see Appendix C) might have to promote in proper seed setting by the overcoming the pollen desiccation and thereby in good seed yields for wet season growing plants.

Table 4 Effects of plant population density on seed yield and yield components of soybean SJ5 cultivar in two different seasons.

PPD (plants m^{-2})	Branch $plant^{-1}$	Pod $plant^{-1}$	Seed pod^{-1}	100-seed weight (g)	Seed yield (kg ha^{-1})
			Wet season		
20 plants m^{-2}	4.14 a[§]	73.7 a	3.09	16.64	2339.02
30 plants m^{-2}	3.98 a	68.88 a	3.03	16.80	2479.26
40 plants m^{-2}	2.33 b	43.45 b	2.97	16.75	2656.57
60 plants m^{-2}	2.26 b	43.75 b	3.00	17.08	2585.18
LSD $_{0.05}$	0.59**	7.31**	NS	NS	NS
			Dry season		
20 plants m^{-2}	2.63 a	31.09 a	2.94	16.08 a	1176.72 b
30 plants m^{-2}	1.83 b	27.04 b	2.86	15.17 a	1310.30 a
40 plants m^{-2}	1.12 c	20.64 c	2.86	15.50 a	1358.90 a
60 plants m^{-2}	0.81 c	17.72 c	2.87	14.92 b	1294.18 a
LSD $_{0.05}$	0.40**	3.26**	NS	1.07*	166.52*

§ Within column, means followed by the same letter are not significantly different at the 0.05 probability level by Fisher's LSD.
*,** Significantly different at the 0.05 and 0.01 probability levels, respectively.
NS = non-significant at the 0.05 probability level.

Table 5 Effects of nitrogen rates on seed yield and yield components of soybean SJ5 cultivar in two different seasons.

N rate (kg ha^{-1})	Branch plant^{-1}	Pod plant^{-1}	Seed pod^{-1}	100-seed weight (g)	Seed yield (kg ha^{-1})
			Wet season		
0	3.05	56.31	3.02	15.80 b[§]	2174.28 c
25	3.43	56.63	3.00	16.59 ab	2483.94 b
50	3.13	59.98	3.02	17.44 a	2558.75 b
75	3.10	56.86	3.09	17.43 a	2843.06 a
LSD $_{0.05}$	NS	NS	NS	0.91**	267.42**
CV$_{(a)}$ %	29.9	17.5	6.5	6.4	19.7
CV$_{(b)}$ %	22.1	15.1	6.3	6.4	12.6
			Dry season		
0	1.71 a	20.72 b	2.77 b	15.17 b	1017.43 c
25	1.23 b	20.20 b	2.87 ab	15.00 b	1134.01 c
50	1.61 ab	26.50 a	2.92 a	15.08 b	1386.14 b
75	1.84 a	29.08 a	2.97 a	16.42 a	1602.52 a
LSD $_{0.05}$	0.40**	3.26**	0.11**	1.07*	166.52**
CV$_{(a)}$ %	15.3	25.8	8.8	8.1	18.0
CV$_{(b)}$ %	29.9	16.0	6.9	8.2	15.3

§ Within column, means followed by the same letter are not significantly different at the 0.05 probability level by Fisher's LSD.
*,** Significantly different at the 0.05 and 0.01 probability levels, respectively.
NS = non-significant at the 0.05 probability level.

3. Seed Protein and Oil Content

Soybean seed protein and oil contents were measured as an estimate of soybean seed composition. To monitor the changes in seed composition during the course of this study, the protein and oil content of seeds were determined by near infrared reflectance spectroscopy analysis.

An analysis of field-grown soybean seeds by near-infrared spectroscopy revealed no significant responses of seed protein content (%) and oil content (%) to PPD (Table 6). It was consistent in different growing seasons ($p > 0.05$). In contrast, Boydak *et al*. (2002) summarized that row spacing and irrigation affected protein and oil contents significantly ($p < 0.01$) in both years. They found that row spacing of 70 cm had the highest protein content, followed by 60, 40, and 50 cm, respectively. Data from the present research showed that seed protein and oil contents were higher in wet season than in dry season by average of 1.6 and 4.5%, respectively. Despite PPD had no significant effect on seed composition in this experiment, it was indicated that accumulation of seed storage components in soybean was influenced by environmental conditions; prevailing during the growing season.

Starter N application significantly affected seed protein content in both seasons (Table 6). Protein content averaged 37.7% in the wet season and 36.05% in the dry season. The wet season growing plants resulted in a 1.65% increase in seed protein content compared with the dry season growing plants. Relative to control, the highest N rate (75 kg ha^{-1}) had significant higher seed protein content by an average of 4% in the wet season, whereas no significant difference was measured in the dry season for this case. Seed oil content ranged from 19.48 to 20% in the wet season and 14.47 to 15.76% in the dry season was not affected statistically by the N fertilization. This is consistent with the reports of Ham *et al*. (1975). Their study in Minnesota observed that preplant broadcast N application increase soybean yield, weight per seed and seed protein, but had no effect on seed oil concentration. This result corroborates the conclusion of Wood *et al*. (1993) that additional fertilizer N does not affect oil content of normal nodulating soybean.

Schmitt *et al*. (2001) also evaluated in-season N applications on soybean in Minnesota. Treatments of urea and polycoated urea applied broadcast and in subsurface bands increased soil nitrate (0- to 30-cm depth) at the full seed stage (R6). According to their results, in-season N application did not increase grain yields but slightly increased seed N removal and grain protein. In general, N treatment effects on soybean seed N and oil concentrations were inconsistent among the different growing seasons. Figure 7 showed negative linear relationships between seed protein and oil content of SJ5 cultivar in the wet and the dry seasons. Since protein and oil content are inversely correlated, the significant increase in protein should be accompanied by a decrease in oil. The protein% of seed in the total seed composition (seed protein plus oil content) accounted for 66% in the wet season and 70% in the dry season.

Table 6 Effects of nitrogen rate and plant population density on seed protein and oil content of soybean SJ5 cultivar at R8 stage in two different seasons.

PPD (plants m^{-2})	N rate (kg ha^{-1})	Wet season		Dry season	
		Protein%	Oil%	Protein%	Oil%
20 plants m^{-2}		37.96	19.82	36.36	15.31
30 plants m^{-2}		38.03	19.80	35.85	15.41
40 plants m^{-2}		37.31	19.82	36.46	14.48
60 plants m^{-2}		37.56	19.59	35.54	15.79
LSD $_{0.05}$		NS	NS	NS	NS
	0	37.08 b[§]	19.68	36.52 a	14.47
	25	37.15 b	20.00	35.22 b	15.74
	50	38.19 a	19.86	35.68 ab	15.76
	75	38.44 a	19.48	36.79 a	15.02
LSD $_{0.05}$		0.99*	NS	1.11*	NS
CV$_{(a)}$ %		4.2	4.9	2.3	10.4
CV$_{(b)}$ %		3.1	3.3	3.7	8.8

§ Within column, means followed by the same letter are not significantly different at the 0.05 probability level by Fisher's LSD.
*,** Significantly different at the 0.05 and 0.01 probability levels, respectively.
NS = non-significant at the 0.05 probability level.

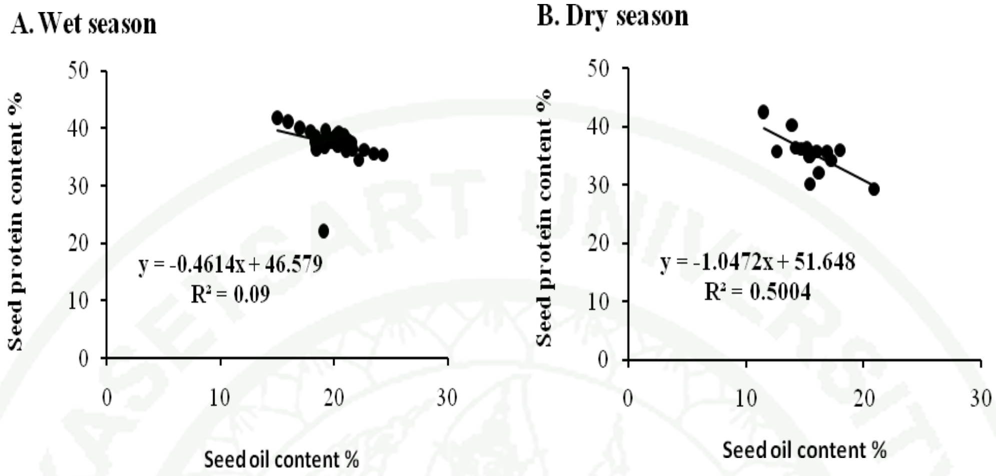

Figure 7 The relationship between seed protein content and oil content of soybean SJ5 cultivar at R8 stage in two different seasons. Values are means of three replications.

4. Correlation between seed yield and some agronomic characters

Table 7 shows correlation coefficient (r) of seed yield and some prominent agronomic growth parameters for soybean SJ5 cultivar grown in two different seasons over the average of four N treatments.

In the wet season, seed yield (kg ha^{-1}) was highly correlated with 100-seed weight (r = 0.74**) among the yield component factors. Pod number per plant and seed number per pod were negative and not significant correlated with yield. Plant growth parameters of SLW-V5 (r = 0.67**), SLW-R5 (r = 0.51*) and TDM-V5 (r = 0.58*) were positively associated with seed yield, while TDM-R5 was not statistically correlated with seed yield. Several studies have shown a positive relationship between duration of seed filling period and yield (Gay *et al.*, 1980; Smith and Nelson, 1986; Shibles and Sundberg, 1998). Conversely, the correlation between seed yield and TDM at the beginning seed filling stage (TDM-R5) in this experiment was not significant (r = -0.15).

In the dry season, seed yield was strongly correlated with seed number per pod (r = 0.68**), but was not correlated with any other yield component data (Table 8). Although yield increased linearly as the pod number per plant (r = 0.44) and 100-seed weight(r = 0.48), there were no significant correlations among them. In addition, the SLW and TDM, which were positively linked to yield, were also not correlated with seed yield. Thus, the characteristic of seed number per pod would be the most important for maximizing seed yield in this season, whereas this character was not associated with seed yield in the wet season. Consistent with the result of Egli (1998), among yield components, seed number per pod was the yield component that accounts for most of the environmental variation in the yield of soybean. This variation provides a general lack of consistency in seed yield performance from season-to-season. Thus, N fertilization and environmental conditions during the growing season appeared to impact overall seed yield in soybean production.

Table 7 Correlation coefficients (r) of seed yield and some agronomic characters of soybean SJ5 cultivar in two different seasons. Values for individual parameters are means of four N rates.

Variables	Seed yields (kg ha^{-1})	
	Wet season	Dry season
SLW-V5	0.67 **	0.20
SLW-R5	0.51 *	0.22
TDM-V5	0.58 *	0.22
TDM-R5	- 0.15	0.41
Pod $plant^{-1}$	- 0.38	0.44
Seed pod^{-1}	- 0.18	0.68 **
100-seed weight	0.74 **	0.48

*, ** Significantly different at the 0.05 and 0.01 probability levels, respectively.

5. Correlation among yield components and some agronomic characters

To evaluate the relationships of yield components and some agronomic growth parameters, Pearson correlation analysis was performed for soybean SJ5 cultivar grown in two different seasons over the average of four N treatments (Table 8).

In the wet season, the data revealed that pod number per plant was strongly correlated with TDM-R5 (r = 0.797**). However, seed number per pod showed a weak linear relationship with TDM-R5 (r = 0.49). It was clearly showed that 100-seed weight was significantly supported by SLW-V5 (r = 0.74**), SLW-R5 (r = 0.61*) and TDM-V5 (r = 0.58*). In this case, seed yield was mainly associated with 100-seed weight (Table 7) and 100-seed weight was also related to SLW and TDM-V5, indicating a stronger dependence upon the amount of TDM-V5 for high seed yield. Despite 100-seed weight was correlated with SLW-R5, it was not linked with TDM-R5 (r = 0.009). Consequently, maximum seed yield resulted by the highest N fertilizer rate suggests that starter N fertilizer application had a positive impact on early plant biomass, and increase in seed yield could be due to an increase in early plant biomass in the wet season.

In the dry season, pod number per plant was strongly linked with all of the plant growth parameters. The rank of order of increasing pod number per plant based on correlation coefficient was TDM-V5 and TDM-R5 > SLW-V5 > SLW-R5. It was also noted that 100-seed weight, an important yield performing factor in the wet season, was highly related to TDM-V5 and TDM-R5.This showed that DM accumulation in plant tissues until the beginning seed filling stage was an important determinant in pod formation and in increasing seed weight of individual plants, while it could not be important in determining seed yield per unit area. Seed number per pod had a positive correlation with SLW-V5 (r = 0.69**), TDM-V5 (r = 0.54*) and TDM-R5 (r = 0.76**). In this season, seed number per pod was a major factor influencing seed yield per unit area (Table 7) and it was strongly associated with TDM-R5 indicating that greater TDM-R5 was necessary to achieve optimum yield potential.

Field studies clearly outlines that yield components such as pod number per plant, seed number per pod and 100-seed weight are likely to be affected by starter N application. The importance of starter N fertilizer application in increasing seed yield is consistent in two different seasons. This increase in seed yield could be due to an increase in plant growth parameters such as SLW and TDM, which responded strongly to the growing season. The optimum PPD for maximizing seed yield per unit area could be defined by lower density in the wet season (20 plants per m^{-2}) than in the dry season (30 plants per m^{-2}). Since accumulation of seed storage components in soybean is influenced by environment prevailing during the growing season, seed protein content shows inconsistent results for N fertilizer treatments under the field conditions. Soybean oil content could not be changed due to increasing N rate or PPD in any growing seasons.

Table 8 Correlation coefficients (r) of yield components and some agronomic characters of soybean SJ5 cultivar in two different seasons. Values for individual parameters are means of four N rates.

Variables	Pod $plant^{-1}$	Seed pod^{-1}	100-seed weight
		Wet season	
SLW-V5	0.11	0.09	0.74 **
SLW-R5	0.09	- 0.01	0.61 *
TDM-V5	0.11	0.03	0.58 *
TDM-R5	0.80 **	0.49	0.01
		Dry season	
SLW-V5	0.74 **	0.69 **	0.54 *
SLW-R5	0.61 *	0.50	0.45
TDM-V5	0.85 **	0.54 *	0.67 **
TDM-R5	0.85 **	0.76 **	0.71 **

*, ** Significantly different at the 0.05 and 0.01 probability levels, respectively.

Hydroponics Experiment (Experiment III)

To evaluate the effect of N fertilizer on soybean seed yield and protein content, a greenhouse experiment was conducted hydroponically. Three soybean cultivars (CKB1, SJ5, CM60) were grown in nutrient culture containing various concentrations of NO_3^- in the forms of KNO_3 and $Ca(NO_3)_2$. Soybean cultivar (SJ5), which was used as a tested cultivar in the previous field experiments, was compared with the two other cultivars (CKB1 and CM60).

1. Plant Growth Analysis

1.1 Specific Leaf Weight (SLW)

Figure 8 indicates significant differences in SLW of soybean cultivars due to increasing NO_3^- concentration in the nutrient solution. At V5 stage, increasing NO_3^- in the nutrient solution resulted significant increases in SLW ranging from 2.3 to 4.1 mg cm^{-2} (Figure 8.A). Although the maximum value was obtained by CKB1 cultivar receiving 10 m*M* NO_3^- level, there was no statistical difference of SLW ($p > 0.05$) gain by increasing NO_3^- level among three cultivars. Relative to control (5 m*M*), the highest NO_3^- level (15 m*M*) showed a significant higher in SLW by 55, 39 and 44 % for CKB1, SJ5 and CM60, respectively. At R5 stage, nutrient treatments had a significant effect on SLW (Figure 8.B). Increasing NO_3^- level from 5 to 10 m*M* maximized the SLW of all cultivars. On average, 75, 56 and 43% higher in SLW was produced by 10 m*M* NO_3^- level for CKB1, SJ5 and CM60, respectively, compared to control level (5 m*M*). However, it was found that SLW was reduced when NO_3^- concentration in the nutrient solution was increased from 10 to 15 m*M*. Cultivars differed significantly ($p<0.01$) in SLW. Among the three cultivars, SJ5 had the highest SLW at every NO_3^- level. The maximum value (6.7 mg cm^{-2}) was obtained by SJ5 cultivar receiving 10 m*M* NO_3^- level.

Dornhoff and Shibles (1976) found a positive relationship between SLW with leaf AP among soybean cultivars and the cultivar differences in SLW were correlated with the variations in leaf thickness. They suggested that higher AP rates in leaves exhibiting greater SLW may reflect the presence of a greater quantity of photosynthetic components contained within the leaves. Furthermore, cultivar differences in leaf AP may have relation to seed yield. According to their findings, SLW appeared closely related to the other leaf attributes and the higher SLW would be needed for the development of a large yield in soybean cultivation. In the present study, it was obvious that increase in SLW due to varying NO_3^- level was likely to be associated with growth stage and cultivar of soybean.

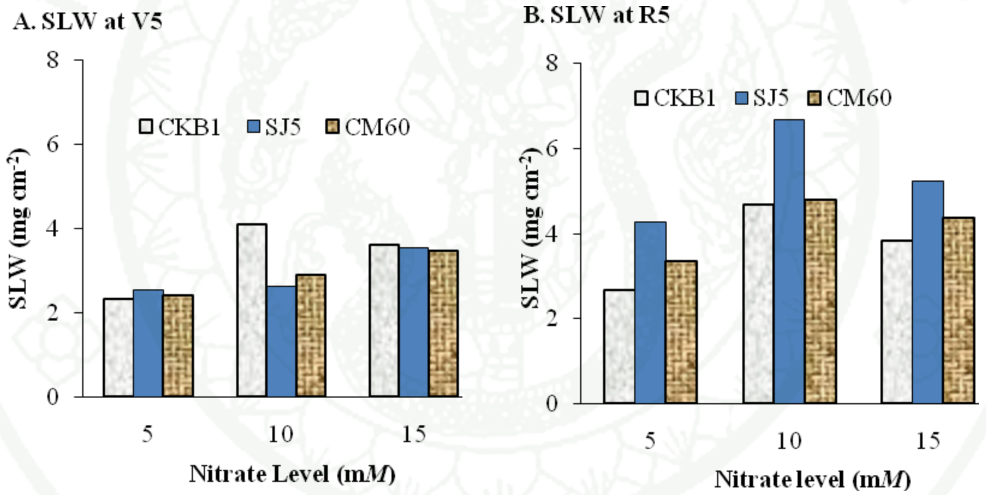

Figure 8 Effects of nitrate levels on specific leaf dry weight (SLW) of three soybean cultivars at V5 and R5 stages.

1.2 Dry Matter (DM) Distribution

1.2.1 Vegetative Stage (V5)

The data presented in Table 9 indicate that DM accumulation in root at vegetative (V5) stage differed significantly ($p < 0.01$) due to increasing NO_3^--N supply. The leaves and stems did not response to N application in DM accumulation.

There was no statistical difference in the whole plant biomass (TDM), whereas plants receiving 15 m*M* NO_3^- accumulated higher DM in the roots than those receiving 5 and 10 m*M* NO_3^-. This result could be due to the lower uptake of N by plants at this stage. Gibson and Harper (1985) reported that the uptake of N is relatively low during seedling and early vegetative growth stages and peak uptake rates per plant occur at the time for early to mid-pod fill under conditions of adequate water supply and continuous NO_3^- availability. Despite NO_3^- levels did not alter TDM, cultivars differences in DM of various plant parts and whole plant were observed at this stage. Among the cultivars, SJ5 showed the minimum accumulation of DM in plant parts, indicating the lowest TDM (2.61 g plant^{-1}).

1.2.2 Reproductive Stage (R5)

At R5 stage, increasing levels of NO_3^- supply had a positive effect on DM accumulation (Table 10). DM accumulation in the whole plant was increased with increasing NO_3^- level from 5 to 10 m*M*, but no more increase was observed when NO_3^- level was added from 10 to 15 m*M*. This indicates that potential accumulation of DM in NO_3^- treated plants was limited by concentration of NO_3^- in the nutrient solution. Vegetative plant parts (i.e. leaf, stem and root) were highly affected by NO_3^- application. In the case of reproductive plant parts (i.e. seed and pod wall), the pod wall DM was significantly different among NO_3^- levels, while NO_3^- application had no significant effect on DM accumulation in the seed. It seems that the amount of DM increased by NO_3^- treatments would be accumulated in the pod wall during the beginning of seed filling period (R5). Assimilates were first allocated to the pod walls and then to the seeds, as pod wall growth began before seed growth.

The higher NO_3^- levels of 10 and 15 m*M* increased TDM by an average of 91% and 99%, respectively, when compared with the 5 m*M* N control. About half of the DM accumulation in the whole plants was allocated to the leaves. Cultivars had a significant different DM accumulation in the reproductive plant parts. At this stage, it was noted that cultivar differences in TDM was not recorded ($p > 0.05$), whereas SJ5 cultivar had the lowest TDM at V5 stage. At R5 stage, SJ5 and CM60 cultivars had a greater DM accumulation in the seed by an average of 104% and 142%, respectively, than did the cultivar of CKB1. In addition, the maximum DM accumulation in the pod wall (11.98 g $plant^{-1}$) was produced by SJ5 cultivar. This indicates that the effect of NO_3^- solution on DM accumulation of soybean largely depends on the cultivars and growth stages.

Table 9 Effects of NO_3^- level on dry matter in each plant part and whole plant of three soybean cultivars at V5 stage. Values for individual treatments are the means for three replicate plants.

Treatment	Dry matter at V5 (g $plant^{-1}$)			
	Leaf	Stem	Root	Whole plant
NO_3^- level				
5 m*M*	2.12	0.46	0.45 b§	3.04
10 m*M*	2.04	0.43	0.46 b	2.94
15 m*M*	2.38	0.55	0.57 a	3.50
$LSD_{0.05}$	NS	NS	0.07**	NS
Cultivar				
CKB1	2.28 a	0.61 a	0.56 a	3.44 a
SJ5	1.92 b	0.28 b	0.42 b	2.61 b
CM60	2.35 a	0.55 a	0.52 a	3.42 a
$LSD_{0.05}$	0.36*	0.13**	0.07**	0.51**
$CV_{(a)}$ %	19.8	14.0	22.7	22.2
$CV_{(b)}$ %	15.9	16.3	14.5	15.8

§ Within column, means followed by the same letter are not significantly different at the 0.05 probability level by Fisher's LSD.
*,** Significantly different at the 0.05 and 0.01 probability levels, respectively.
NS = non-significant at the 0.05 probability level.

Table 10 Effects of NO_3^- level on dry matter in each plant part and whole plant of three soybean cultivars at R5 stage. Values for individual treatments are the means for three replicate plants.

Treatment	Dry matter at R5 (g plant^{-1})					
	Leaf	Stem	Root	Seed	Pod wall	Whole plant
NO_3^- level						
5 m*M*	19.25 b[§]	9.15 b	4.21 b	0.65	4.37 b	37.62 b
10 m*M*	36.80 a	17.25 a	8.37 a	0.85	8.72 a	72.00 a
15 m*M*	39.31 a	16.26 a	7.18 a	1.12	10.92 a	74.80 a
LSD $_{0.05}$	8.63**	3.72**	1.50**	NS	2.51**	14.17**
Cultivar						
CKB1	30.73	15.79	6.99	0.48 b	5.17 b	59.16
SJ5	35.21	13.34	6.79	0.98 a	11.98 a	68.29
CM60	29.43	13.54	5.99	1.16 a	6.86 b	56.97
LSD $_{0.05}$	NS	NS	NS	0.52*	2.51**	NS
$CV_{(a)}$ %	14.7	11.1	26.0	22.3	23.2	25.1
$CV_{(b)}$ %	13.2	12.7	11.1	18.9	15.3	22.4

§ Within column, means followed by the same letter are not significantly different at the 0.05 probability level by Fisher's LSD.
*,** Significantly different at the 0.05 and 0.01 probability levels, respectively.
NS = non-significant at the 0.05 probability level.

1.3 Relationship between organ DM and TDM at R5

There were significant positive relationships between organ DM and TDM of three soybean cultivars treated with various NO_3^- levels (Figure 9). The rank of order of increasing biomass on regression line basis was leaf > stem > root > pod. This is consistent with the results of previous field experiments, but the coefficient of determination of stem DM (R^2 = 0.79) was not as strong as those under the field conditions (Figures 5.B and 6.B). For vegetative plant organs, DM accumulation in the leaves was strongly related with TDM in both fields and greenhouse experiments, suggesting that the leaves were the main source for increasing TDM. For reproductive organs, DM accumulation in the pod was closely related with TDM ($R^2 = 0.61$) (Figure 9.D) under the greenhouse condition, while it was weakly linked with TDM under the field conditions (Figures 5.D and 6.D). Thus, it could be suggested that the effect of N fertilizer on the pod formation at R5 stage was apparently observed from plants cultured in the nutrient solutions.

1.4 Shoot/root ratio at V5 and R5

The biomass partitioning at V5 stage was expressed in the form of shoot/root ratio (Figure 10). A comparison of the three soybean cultivars on the basic of shoot/root showed that variation between the cultivars were mainly associated with level of nutrition. CKB1 had the same values of shoot/root among the various NO_3^- levels. SJ5 cultivar attained a maximum value of 6.7 at the 10 m*M* and a minimum value of 4.8 at the highest level (15 m*M*). However, CM60 had the highest ratio (6.6) at the lowest level of nutrition. All cultivars did not have a largest shoot/root ratio at the highest level, and there was no significant difference between the ratios of any of the cultivars.Thus, the effect of the highest level of nutrient solution was to increase the root bulk of each cultivar, but not to bring out varietal differences in response to NO_3^- level. Van der Werf and Nagel (1996) reported that plants growing on low NO_3^- supply typically display a higher root-to-shoot ratio than plants adequately fed with this nutrient. In contrast, increase in NO_3^- nutrition leading to a lower shoot/root ratio, due mainly to increase root growth was observed at vegetative stage.

At R5 stage, NO_3^- solution had a considerable effect on the shoot/root ratio (Figure 11). CM60 had the higher ratio (15.15) than either of the other two cultivars at the lowest NO_3^- level. This cultivar produced the largest shoot biomass under the lowest NO_3^- regime at both growth stages. On the other hand, CKB1 and SJ5 showed the greatest values of shoot/root ratio at the highest NO_3^- level. These two cultivars produced the largest shoot biomass under the highest NO_3^- regime. This indicated that increase in NO_3^- solution produced the higher shoot/root ratio at reproductive stage.

Kasperbauer and Hunt (1992) reported that shoot/root relationships of soybeans were influenced by genetics and environmental conditions that exist during plant growth. Similarly, the present results indicated that variation in shoot/root ratios of the three tested cultivars were inconsistent with plant age due to the various levels of NO_3^- solution.

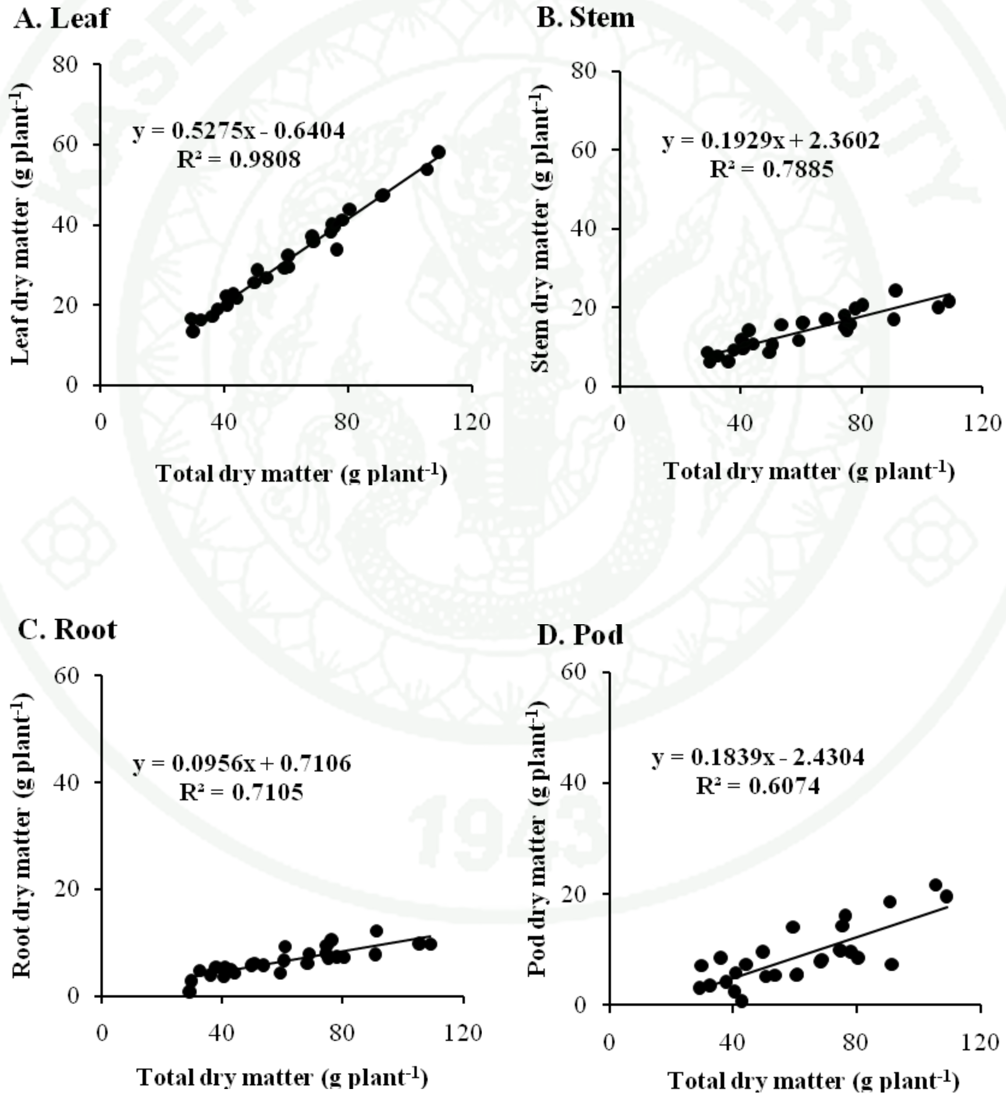

Figure 9 The relationship between organ dry matter and total plant biomass of three soybean cultivars at R5 stage.

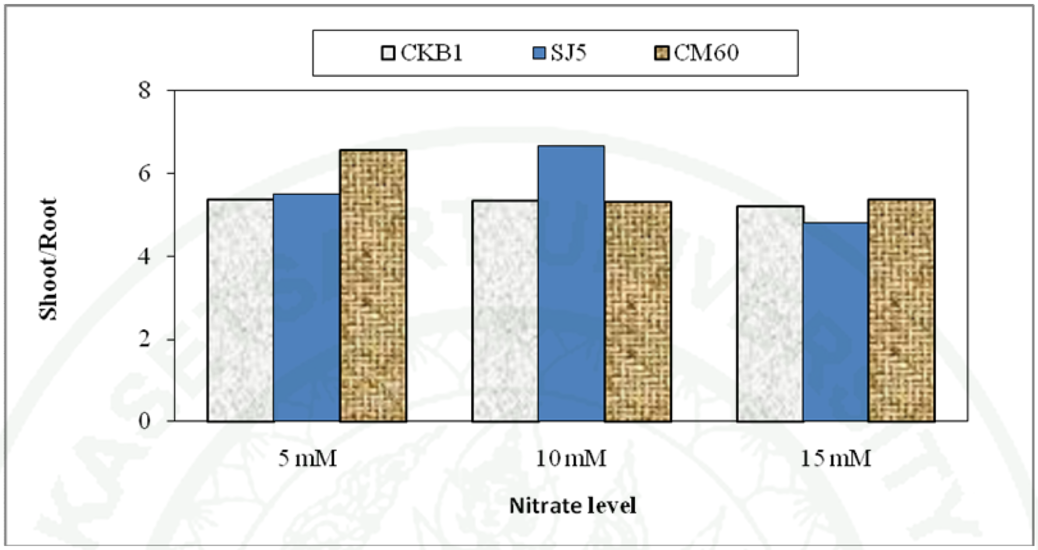

Figure 10 Effects of nitrate levels on shoot/root ratio of three soybean cultivars at V5 stage.

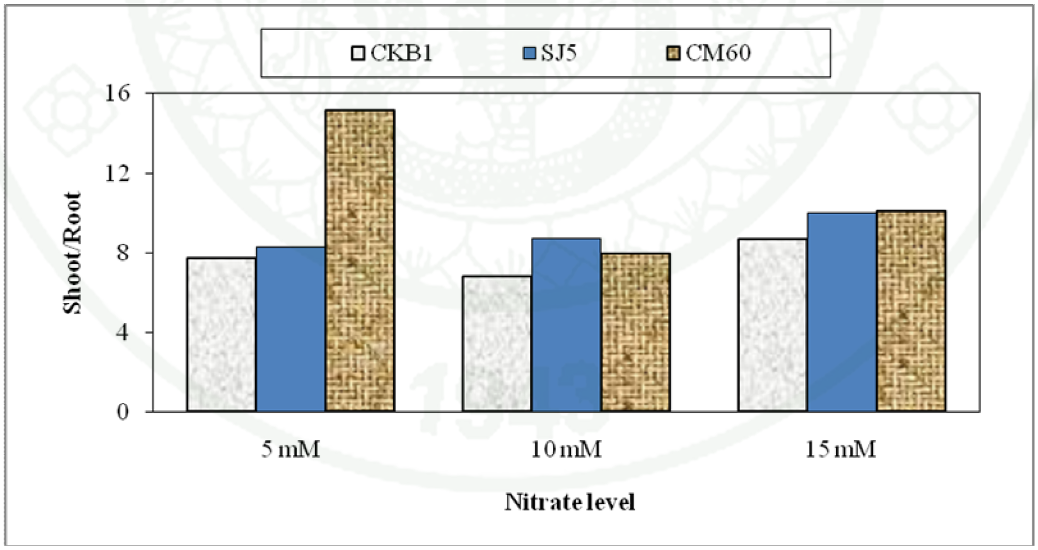

Figure 11 Effects of nitrate levels on shoot/root ratio of three soybean cultivars at R5 stage.

2. Carbon and N accumulation in the leaves at R5

The C content in the leaves of all three soybean cultivars at R5 stage was significantly different ($p < 0.05$) due to varying NO_3^- levels (Table 11). Average across the cultivars, the highest C content (43.4%) was observed in the leaves of plant treated with 15 m*M* NO_3^- level. Plants receiving 10 m*M* accumulated higher C in leaves compared to 5 m*M* (control) by an average of 3 and 1% for CM60 and SJ5 cultivars, respectively, while CKB1 cultivar had no significant increase in the leaf C content at 10 m*M* NO_3^- level. The maximum value (44.2%), however, was observed from CKB1 cultivar treated with 15 m*M* NO_3^-. SJ5 had a positive linear increase in the leaf C content with increasing level of NO_3^- treatments. CM60 showed slightly decrease in the leaf C content when NO_3^- concentration was increased from 10 to 15 m*M*.

The N content in the leaves at R5 stage differed significantly ($p< 0.01$) due to varying NO_3^--N level (Table 11). The accumulation of N in the leaf increased with concurrent increasing levels of NO_3^- in the solution. The maximum value (4.3%) was observed at the highest NO_3^- level. The leaf N content was linearly increased with increasing NO_3^- level from 5 to 15 m*M*. The increment was 82, 37 and 32% for SJ5, CKB1 and CM60, respectively. On average, SJ5 cultivar receiving 15 m*M* NO_3^- had a maximum N content (3.6%) among the three cultivars. These findings are in agreement with the result of Hanway and Weber (1971) which indicated that the soybean cultivars differed greatly amongst themselves in absorption of N under varied N fertilizer supply. Hesketh *et al*. (1981) also reported the differences in total leaf protein content of soybean cultivars and are positively correlated to single leaf apparent photosynthesis.

3. Leaf chlorophyll content

The leaf chlorophyll content was determined throughout the plant cycle at weekly intervals. The first measurement was undertaken at 15 days after transplanting (DAT), V5 stage, and the last at 50 DAT, R5 stage. Chlorophyll contents were examined with SPAD-502 meter readings. The SPAD-502 chlorophyll meter, is a nondestructive, hand held meter available for measurement of the green color intensity in crop leaves which is directly related to leaf chlorophyll.

At V5 stage, 15 DAT, chlorophyll content in the leaves of all cultivars was not significantly different by treating NO_3^- nutrition (Table 12). A week after V5 stage, significant differences in leaf chlorophyll content were observed at each measurement. Chlorophyll content linearly increased with increasing NO_3^- level. The highest level (15 m*M*) maximized the leaf chlorophyll content during pod setting period, 22 to 36 DAT. At R5 stage, 50 DAT, the maximum chlorophyll content of 43.11 was achieved in the leaves of plants receiving 15 m*M* NO_3^- treatment, but it was not statistically different from the values resulted by 10 m*M* NO_3^- treatment. The mean values of chlorophyll content were increased 38 and 46% when NO_3^- concentration was increased from 5 to 10 and 15 m*M*, respectively. This suggests that N is frequently considered the more important into essential elements, due participation in biosynthesis of molecules as well as it contributes directly in the formation of chlorophylls that will be used in fundamental processes to plant growth and development. Delgado *et al*. (1994) reported that N deficiency in the plants has been shown to cause a decrease in the levels of photosynthetic structural components such as chlophyll. Cultivars showed significant differences in chlorophyll content only at 22 DAT (Table 12). The chlorophyll content of all cultivars increased with crop ontogeny due to increasing NO_3^- level. The chlorophyll content of soybean leaves was low early in the growing season; reach to peak value at the beginning of seed filling stage (R5). The results of the present study indicate that chlorophyll content in the leaves of soybean cultivars are likely to be promoted by varying NO_3^- levels.

Table 11 Effects of NO_3^- level on carbon and nitrogen content in the leaves of three soybean cultivars at R5 stage. Values for individual treatments are the means for three replicate plants.

NO_3 level	Cultivar	R5 stage	
		Leaf C%	Leaf N%
5 mM	CKB1	42.871	3.239
	SJ5	41.597	2.628
	CM60	42.098	2.791
10 mM	CKB1	42.869	3.550
	SJ5	42.011	3.250
	CM60	43.487	3.235
15 mM	CKB1	44.203	4.444
	SJ5	43.001	4.791
	CM60	42.853	3.696
5 mM		42.189 b§	2.886 c
10 mM		42.789 b	3.345 b
15 mM		43.352 a	4.310 a
LSD $_{0.05}$		0.697*	0.235**
	CKB1	43.314 a	3.745 a
	SJ5	42.203 b	3.556 a
	CM60	42.813 b	3.241 b
LSD $_{0.05}$		0.697*	0.235**
$CV_{(a)}$ %		1.6	10.1
$CV_{(b)}$ %		1.6	6.5

§ Within column, means followed by the same letter are not significantly different at the 0.05 probability level by Fisher's LSD.

*,** Significantly different at the 0.05 and 0.01 probability levels, respectively.

NS = non-significant at the 0.05 probability level.

Table 12 Effects of NO_3^- level on chlorophyll content of three soybean cultivars. Values for individual treatments are the means for three replicate plants.

NO_3^- level	Cultivar	Chlorophyll Unit (SPAD)					
		15	22	29	36	43	50
		(Days after transplanting)					
5 m*M*	CKB1	28.80	32.83	28.27	24.27	26.37	29.70
	SJ5	24.97	30.13	27.80	28.27	27.23	27.60
	CM60	28.37	28.27	30.17	30.17	31.07	31.33
10 m*M*	CKB1	28.33	31.87	33.17	36.60	40.27	40.23
	SJ5	28.57	30.30	32.03	38.17	39.83	39.73
	CM60	26.13	32.83	32.00	34.63	39.57	42.77
15 m*M*	CKB1	29.10	34.07	38.53	39.67	40.60	44.03
	SJ5	29.13	31.80	36.10	40.17	41.70	43.63
	CM60	27.70	32.03	36.37	37.33	40.33	41.67
5 m*M*		27.38	30.41 b[§]	28.74 c	27.57 c	28.22 b	29.54 b
10 m*M*		27.68	31.67 ab	32.40 b	36.47 b	39.89 a	40.91 a
15 m*M*		28.64	32.63 a	37.00 a	39.06 a	40.88 a	43.11 a
$\text{LSD}_{0.05}$		NS	1.59*	2.042**	2.32**	2.30**	3.83**
	CKB1	28.74	32.92 a	33.32	33.51	35.74	37.99
	SJ5	27.56	30.74 b	31.98	35.53	36.26	36.99
	CM60	27.40	31.04 b	32.84	34.04	36.99	38.59
$\text{LSD}_{0.05}$		NS	1.59*	NS	NS	NS	NS
$\text{CV}_{(a)}$ %		4.01	6.49	4.81	1.8	4.58	4.72
$\text{CV}_{(b)}$ %		7.35	4.9	6.08	6.59	6.17	9.85

§ Within column, means followed by the same letter are not significantly different at the 0.05 probability level by Fisher's LSD.
*,** Significantly different at the 0.05 and 0.01 probability levels, respectively.
NS = non-significant at the 0.05 probability level.

4. Yield components and seed yield

4.1 Seed yield response to NO_3^- application

Nitrogen application had a positive influence on yield components and seed yield per plant (Table 13). The greatest seed yield (48.12 g $plant^{-1}$) was obtained from plants receiving 15 m*M* NO_3^-, while the lowest (17.72 g $plant^{-1}$) was obtained from plants receiving 5 m*M* NO_3^- (control). The branch number per plant was not significantly different between 10 and 15 m*M* NO_3^- treatments, hence the greatest seed yield per plant receiving 15 m*M* was mainly associated with higher pod number per plant. This is consistent with the result of Board and Tan (1995) implicating pod number as an important factor influences on soybean yield.

Cultivars gave significant differences in the final seed yield (Table 13). For all cultivars, seed yield per plant was increased with increasing NO_3^- level. It was clearly noted that the highest seed yield was not obtained by SJ5 cultivar at the lowest NO_3^- level (control); however, SJ5 had the highest seed yield per plant with increasing NO_3^- level from control to 10 and 15 m*M*. It seems to be associated with cultivar differences in the pod number per plant due to varying NO_3^- supply. In the case of pod per plant, average number of pod per plant was positively increased with increased NO_3^- level from 5 to 15 m*M* for all cultivars, while it was not statistically different from each cultivar. Among the three cultivars, SJ5 produced the lowest number of pod (78.33) at 5 m*M* NO_3^- level, but the highest numbers of pod per plant were observed from SJ5 cultivar when NO_3^- level was increased. As a result, SJ5 had the highest seed yield per plant, averaging 41.43 g $plant^{-1}$ compared with the remaining two cultivars. This suggests a positive effect of NO_3^- treatment on pod number per plant would be a major component influencing seed yield of the soybean cultivar,

4.2 Relationship between seed yield and leaf N content at R5

A positive relationship between seed yield and leaf N content at R5 was observed in all of the cultivars (Figure 12). The leaf N content was linearly related with seed yield due to application of NO_3^- treatments. Shibles and Sundberg (1998), consistent with the present study, reported a positive relationship between leaf N content during the seed filling period (R5) and final seed yield. They proposed that leaf N content at R5 was more important than percentage of N mobilized in establishing the amount of leaf N mobilized. Kumudini *et al*. (2002) also found a stronger dependence upon leaf N accumulation in the leaves at R5 for the yield improvement of the short-season soybeans. Since proteins are initially accumulated in the leaves and the roots, and contribute to biomass production before breakdown and redistribution to reproductive plant parts (Ohyma, 1983), N accumulation by leaf tissues during seed growth is an important determinant of seed yield.

SJ5 cultivar, representing the highest seed yield in the present study, exhibited the closet relationship ($R^2 = 0.61$) between leaf N at R5 and seed yield rather than the other two cultivars. These results are supported by Loberg *et al*. (1984) reported that cultivars differ in the amount of N accumulated in vegetative tissues and in the proportion of vegetative N mobilized for seed growth. In general, soybean yield correlates closely to total N in the plant because of the large amount of N accumulation in the seed. A number of studies (Jeppson *et al*., 1978; Spaeth and Sinclair, 1983; Loberg *et al*., 1984; Leffel *et al*., 1992) indicated that there were genotypic variations in N accumulation and its partitioning to the seeds. The result of the present study suggests that the leaf N at R5 would be important and needed for the development of a large yield, but the degree to which the plant depends upon N accumulation in the leaves for seed yield likely varies according to the cultivars.

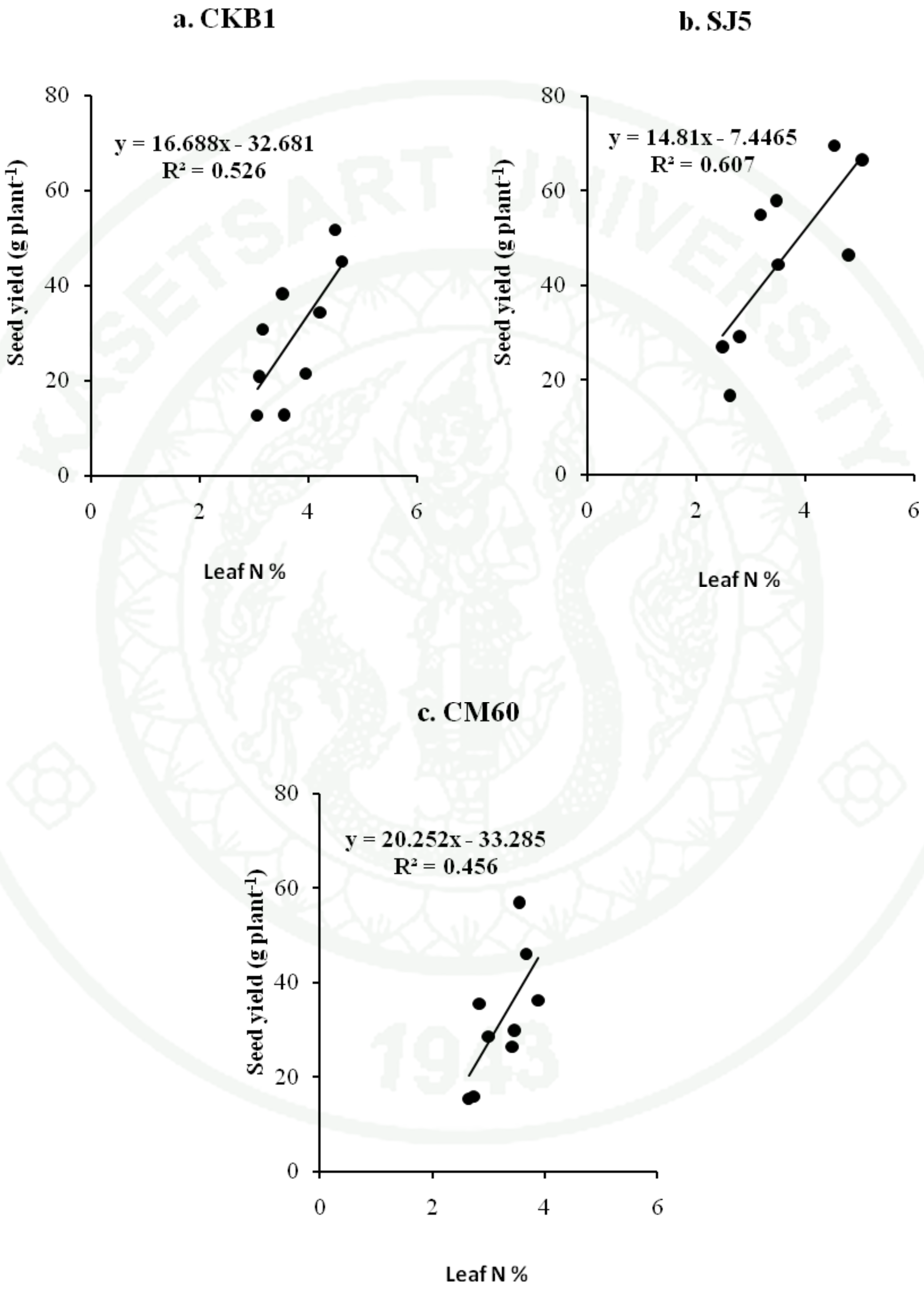

Figure 12 Relationship between seed yield and leaf nitrogen content at R5 of three soybean cultivars: a) CKB1, b) SJ5 and c) CM60, averaged over the various NO_3^- levels.

Table 13 Effects of NO_3^- level on yield components and seed yield of three soybean cultivars. Values for individual treatments are the means for three replicate plants.

NO_3 level	Cultivar	Branch plant^{-1}	Pod plant^{-1}	Seed yield (g plant^{-1})
5 mM	CKB1	5.67	86.00	15.50
	SJ5	4.00	78.33	17.67
	CM60	4.00	102.67	19.98
10 mM	CKB1	5.33	130.67	30.19
	SJ5	6.33	175.67	52.44
	CM60	6.33	148.33	30.62
15 mM	CKB1	6.33	180.00	43.74
	SJ5	6.33	190.00	54.19
	CM60	5.67	182.67	46.44
5 mM		4.56 b§	89.00 c	17.72 c
10 mM		6.00 a	151.56 b	37.75 b
15 mM		6.11 a	184.22 a	48.12 a
LSD $_{0.05}$		1.09*	19.40**	5.12**
	CKB1	5.78	132.22	29.81 b
	SJ5	5.56	148.00	41.43 a
	CM60	5.33	144.56	32.35 b
LSD $_{0.05}$		NS	NS	5.12**
CV$_{(a)}$ %		9.9	13.0	18.2
CV$_{(b)}$ %		9.6	6.7	7.2

§ Within column, means followed by the same letter are not significantly different at the 0.05 probability level by Fisher's LSD.
*,** Significantly different at the 0.05 and 0.01 probability levels, respectively.
NS = non-significant at the 0.05 probability level.

5. Carbon content in the reproductive plant parts

Application of NO_3^- solution had negative effect on seed C percentage (Table 14) at the beginning seed filling stage (R5). The maximum C content (45.6%) was accumulated in the seed receiving 5 m*M* NO_3^-. The seed C percentage was decreased with increasing NO_3^- concentration from 5 to 10 m*M* and no more decrease was found when NO_3^- concentration was increased from 10 to 15 m*M*. At maturity stage (R8), the C content of the seed ranged from 49.6 to 50.5% and no statistically differences were observed among NO_3^- treatments. The highest accumulation of C percentage was located in the pod wall receiving 15 m*M* NO_3^- at both growth stages. Relative to beginning seed filling stage, mature seed increased C percentage receiving 5, 10 and 15 m*M* NO_3^- by an average of 9, 17 and 18%, respectively. All cultivars tested in the present experiment showed significant differences in the seed C content at R5 supplied by NO_3^- fertilizer. The largest percentage of C (44.72%) was observed in the seed of CKB1 cultivar. However, there was no significant effect on the seed C content at R8 among the cultivars. SJ5 cultivar had a significant highest percentage of the pod wall C (40%) at R8 stage.

6. Protein content in the reproductive plant parts

6.1 Protein content in the pod wall and seed at R5 and R8

The N contents in the reproductive plant parts (pod wall and seed) at R5 and R8 stages were determined with an Elementary CHN Autoanalyzer (TruSpec CHN, LECO. Corp., St. Joseph, Michigan, USA). The trend of variation in the protein content was similar to that of N content due to conversion by multiplying the N content in the seeds with 6.25.

At R5, the protein content in the pod wall of the three soybean cultivars differed significantly ($p< 0.01$) due to varying NO_3^- concentration (Table 15). Plants supplied with 15 m*M* NO_3^- had the highest protein content in the pod wall (15.06%). However, the seed protein content was not statistically different among the NO_3^-

treatments. Cultivars differed in both pod wall and seed protein contents. SJ5 cultivar had the highest pod wall protein content (14.64%) and CM60 had the highest seed protein content (45.01%).

Conversely, the protein content in the mature seed (R8) exhibited significant response to NO_3^- treatment but no response of the pod wall protein content among NO_3^- levels (Table 14). The maximum protein content (47.06%) in the seed was produced by 15 m*M* NO_3^- treatment which was 15.5% higher than that receiving 5 m*M* (control) but statistically similar to that of 10 m*M* NO_3^- treatment. At maturity, 10 and 15 m*M* NO_3^- treatments increased the seed protein content by an average of 10%. The control treatment slightly reduced the seed protein content when the seed was mature. No cultivar differences in terms of the pod wall and seed protein contents at the R8 stage were recorded. For each cultivar, 15 m*M* NO_3^- treatment increased protein content in the mature seed of CKB1, SJ5 and CM60 by an average of 10.7 %, 15.4% and 20.9%, respectively, compared to 5 m*M* NO_3^- treatment.

Nitrogen regulation of storage protein subunit levels of soybean seeds was evaluated by many researchers (Ohtake *et al*., 1997; Paek *et al*., 1997; Nakasathien *et al*., 2000). They observed that the change in protein composition of soybean seed with change in protein concentration as influenced by nitrogen source supplied. Imsande (2001) also found that protein synthesis in soybean was influenced by elements such as P, K, N, and the optimum applications of these elements increase the storage proteins. The findings of the present study agreed with the previous results that 15 m*M* NO_3^- level increased protein content in the seeds from 42.9 to 47.1%, but decreased in the pod walls with crop ontogeny.

Table 14 Effects of NO_3^- level on carbon content in pod wall and seed of three soybean cultivars. Values for individual treatments are the means for three replicate plants.

Treatment	R5 (Carbon %)		R8 (Carbon %)	
	Pod wall	Seed	Pod wall	Seed
NO_3^- level				
5 m*M*	39.91 c[§]	45.62 a	38.87 c	49.636
10 m*M*	40.42 b	42.50 b	39.47 b	49.792
15 m*M*	40.91 a	42.66 b	40.31 a	50.488
LSD $_{0.05}$	0.39**	0.79**	0.39**	NS
Cultivar				
CKB1	40.38	44.72 a	39.26 b	50.796
SJ5	40.31	43.30 b	40.00 a	49.645
CM60	40.55	42.78 b	39.39 b	49.474
LSD $_{0.05}$	NS	0.79**	0.39**	NS

§ Within column, means followed by the same letter are not significantly different at the 0.05 probability level by Fisher's LSD.
*,** Significantly different at the 0.05 and 0.01 probability levels, respectively.
NS = non-significant at the 0.05 probability level.

6.2 Relationship between N content in the mature seed and leaf N at R5

Leaf N content at R5 was positively related with N content in the mature seed of soybean cultivars (Figure 13). Coefficients of determination indicated that CM60 (R^2 = 0.62) was a stronger dependence upon the leaf N for the seed N content. Cultivars of CKB1 and SJ5 showed a linear R^2, but the coefficients of determination were not as strong as those for the seed yield (Figure 12). It seems that the requirement of N for the seed yield and protein synthesis of soybean may be supplied by N content in the leaf tissue, and the amount of the requirement depends on the cultivar. Sinclair and de Wit (1976) postulated that the high demand for N by the high protein grain crops, such as soybean, cannot be satisfied by daily N accumulation rates and therefore N must be remobilized from vegetative tissue. Since leaf N content was found to be correlated with N remobilized from vegetative tissue (Shibles and Sundberg, 1998), the results in the present study suggests that leaf N content could be defined as an important determinant of soybean yield and quality.

Table 15 Effects of NO_3^- level on protein content in the pod wall and seed of three soybean cultivars. Values for individual treatments are the means for three replicate plants.

NO_3 level	Cultivar	R5 (Protein %)		R8 (Protein %)	
		Pod wall	Seed	Pod wall	Seed
5 mM	CKB1	12.05	49.28	2.34	40.96
	SJ5	11.01	40.21	3.77	41.87
	CM60	9.70	37.55	2.88	39.34
10 mM	CKB1	11.50	42.29	2.81	44.48
	SJ5	13.67	42.85	1.56	48.96
	CM60	10.39	39.32	4.99	43.40
15 mM	CKB1	13.53	43.48	2.74	45.33
	SJ5	19.18	44.84	2.12	48.31
	CM60	12.47	40.23	3.98	47.55
5 mM		10.92 b§	42.35	2.99	40.73 b
10 mM		11.85 b	41.49	3.12	45.61 a
15 mM		15.06 a	42.85	2.94	47.06 a
LSD $_{0.05}$		1.04**	NS	NS	3.05**
	CKB1	12.36 b	45.01 a	2.63	43.59
	SJ5	14.62 a	42.64 b	2.48	46.38
	CM60	10.85 c	39.03 c	3.95	43.43
LSD $_{0.05}$		1.04**	1.76**	NS	NS
$CV_{(a)}$ %		16.6	6.2	12.1	7.9
$CV_{(b)}$ %		27.9	14.1	22.1	8.7

§ Within column, means followed by the same letter are not significantly different at the 0.05 probability level by Fisher's LSD.
*,** Significantly different at the 0.05 and 0.01 probability levels, respectively.
NS = non-significant at the 0.05 probability level.

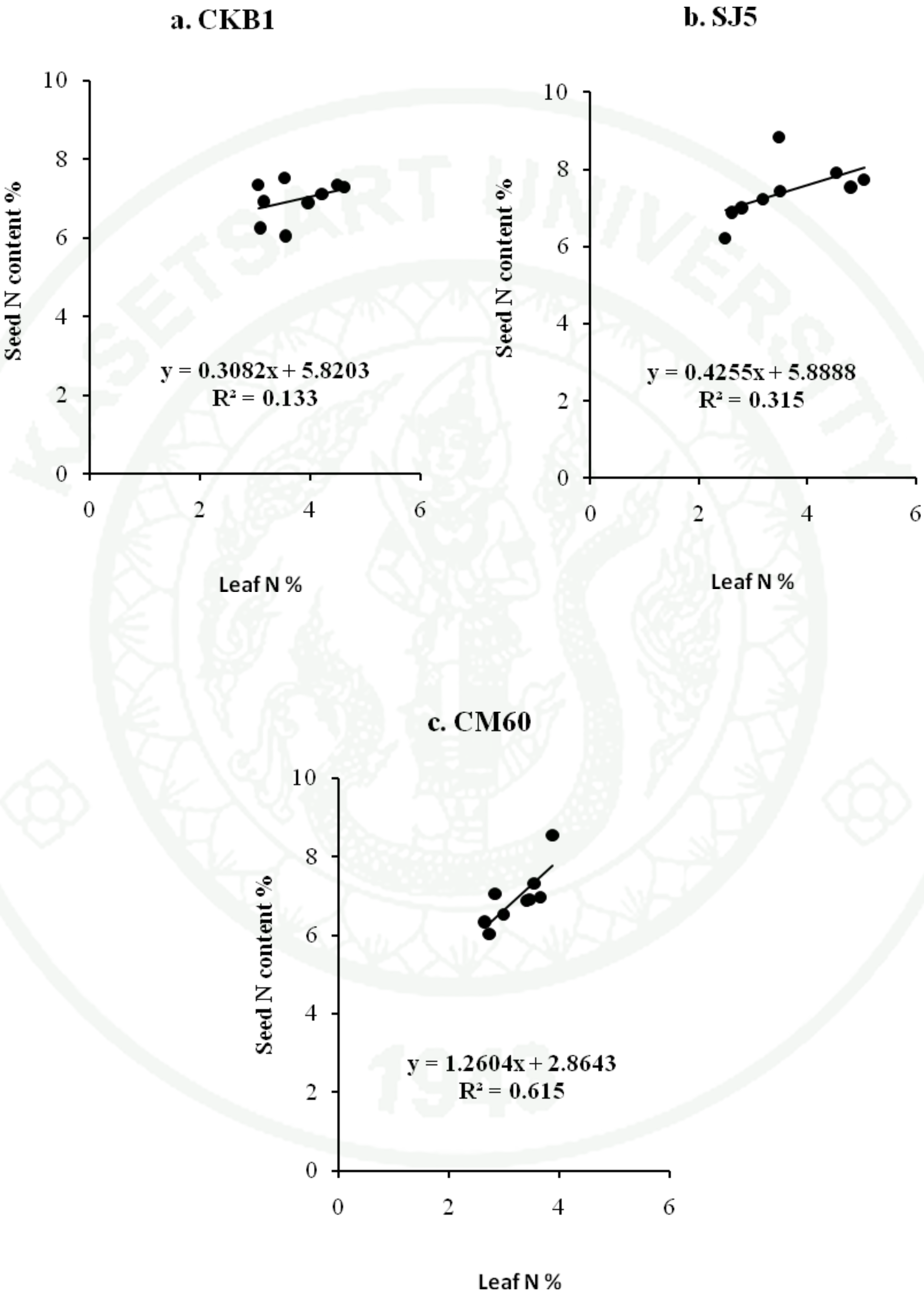

Figure 13 Relationship between nitrogen content in the mature seed and leaf nitrogen content at R5 of three soybean cultivars: a) CKB1, b) SJ5 and c) CM60, averaged over the various NO_3^- levels.

7. Correlation coefficients (r) of seed yield, protein content and some agronomic characters

Table 16 shows Pearson correlation coefficients for the relationship of seed yield, protein content and some agronomic traits. The correlation coefficients were not significant between SLW at V5 and seed yield (r = 0.51), and seed protein content (r = 0.52). Conversely, SLW at R5 was significantly associated with both seed yield (r = 0.71*) and seed protein content (r = 0.78*), indicating a strong dependence upon SLW at R5 for seed yield and protein content. Hesketh *et al*. (1981) has been observed a positive relationship between leaf net photosynthetic rate and SLW among soybean cultivars. Since high SLW may increase leaf net photosynthetic rate (Thompson *et al*., 1995), it was postulated that SJ5 cultivar among the observed cultivars in the present study may have the possibility of the contribution of a higher leaf net photosynthetic rate during the seed development to a higher seed yield. It was evident that SJ5 cultivar had a maximum SLW in both growth stages (Figure 8) resulting the highest seed yield (Table 13).

Regarding the DM accumulation, TDM at V5 was negative and not significant correlated with seed yield and seed protein content (Table 15). The coefficients were not different between seed yield and seed protein content (r = -0.11). On the other hand, there were significant relationships of TDM at R5 with seed yield and seed protein content. The correlation coefficient (r = 0.88**) indicated that TDM at R5 was strongly associated with seed yield compared to the correlation with seed protein content(r = 0.86*). Thus, TDM accumulation at R5 stage seems to provide for seed formation and quality of soybean.

Table 16 Correlation coefficients (r) of seed yield, seed protein content and some agronomic characters of three soybean cultivars.

Variables	Seed yield (g plant^{-1})	Seed protein (%)
SLW-V5	0.51	0.52
SLW-R5	0.71	0.78*
DM-V5	- 0.11	- 0.11
DM-R5	0.88**	0.86*

*, ** Significantly different at the 0.05 and 0.01 probability levels, respectively.

CONCLUSION

To observe the effect of N fertilizer on the response of soybean under the field condition, SJ5 cultivar was tested as a recommended Thai-cultivar. The results of the previous field experiments were evaluated with those of greenhouse experiment.

In field experiments, starter N fertilizer application had a positive impact on seed yield of soybean SJ5 cultivar in both seasons. The highest N rate (75 kg ha^{-1}) increased seed yield by an average of 31% for the wet season and 57% for the dry season when compared with control (0 kg ha^{-1}). This increase in seed yield response to N fertilizer treatment was due primarily to the largest number of pod per plant and 100-seed weight. In addition, the highest rate of N application induced the plant to the highest SLW and DM accumulation in each plant part. Among the plant organs, leaves were responsible for the highest amount of TDM, indicating a significant yield difference between two different seasons. Seed protein content showed inconsistent results for two seasons. In the wet season, the highest N rate (75 kg ha^{-1}) had significant higher seed protein content by an average of 4% compared to the control treatment, whereas no significant difference was measured between the treatments of 50 and 75 kg ha^{-1}. In the dry season, the N fertilizer treatment also showed a significant effect on seed protein content but the increment of seed protein percent between the highest and control N rates were not statistically different. Thus, environmental conditions during the growing season appeared to impact growth parameters and seed quality under the field conditions.

Based on the results of field studies, it can be concluded that the N fertilizer treatment had a consistent effect on seed yield of SJ5 cultivar at any arrangements of PPD during growing seasons. However, PPD seems to differ overall seed yield, with the wet season having the same yield per unit area for all treatments and the dry season having the highest seed yield resulted by PPD of 30 plants m^{-2}. There was no statistical response of seed oil content due to PPD or starter N application in any growing seasons. In the field experiments, soil samples were collected and analyzed just before and after conducting research to determine the amount of residual soil organic matter (OM). There was no difference in residual soil OM in any of the growing seasons with the average level of moderate (did not analyze N% in it), therefore, it is likely that the response of soybean was due to fertilizer application not residual soil OM.

In the greenhouse experiment, NO_3^- application had a positive impact on seed yield of all tested soybean cultivars. Increasing NO_3^- concentration in the nutrient solution resulted in significant increase in DM of each plant part and whole plant at R5 stage, and most of the increase was associated with a greater leaf DM. The maximum seed yield was obtained from the highest NO_3^- level (15 m*M*) mainly associated with the higher number of pod per plant. Moreover, the leaf N content at R5 and leaf chlorophyll content were significantly influenced by NO_3^- application and the maximum values of all three cultivars were observed at the highest NO_3^- level. The existence of a linear relationship between final seed yield and leaf N content at R5 (Fig. 12) confirmed the importance of N accumulation in the leaves at R5 for final seed production. Increase in the protein content of mature seed was observed in the limited level of NO_3^- because the increment resulted by 10 and 15 m*M* was statistically the same value (10%), compared to control. Thus, 10 m*M* NO_3^- could be identified as an appropriate concentration for seed protein production. Superior N accumulation in the leaf tissue at R5 would be more closely related to seed yield than seed N content (Figures. 12 and 13). Cultivars were likely to differ in protein percentage of reproductive plant parts at R5 than at R8 due to varying NO_3^- treatments. Among three soybean cultivars, SJ5 gave the highest response in biomass, leaf N content at R5 and seed yield to increased application of NO_3^- level. The greater seed yield of SJ5 cultivar would be accompanied with greater DM production in the leaves at R5 and the higher C accumulation in the pod wall at R8.

Collectively, results of the all experiments suggest that seed yield of soybean could be increased by N fertilizers in the forms of urea and NO_3^-, and the increment was mainly associated with the extent of TDM and N content in the leaves at R5 stage of the cultivars. The optimum level of N fertilizer for increasing protein content in the mature seed was lower than that for maximizing seed yield of the observed cultivars.

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APPENDIX

Appendix A Selected soil characteristics of research sites, National Corn and Sorghum Research Center, Experiment I.

	pH	Soil texture	OM	P	K	Ca	Mg
				Before Experiment			
Control	6.8	Clay	M	VH	L	H	H
				After Experiment			
N Rate							
0 kg ha^{-1}	6.7	Clay	M	VH	VH	H	H
25 kg ha^{-1}	7.3	Clay	M	VH	VH	H	H
50 kg ha^{-1}	7.4	Clay	M	VH	VH	H	H
75 kg ha^{-1}	7.4	Clay	M	VH	VH	H	H

Appendix B Selected soil characteristics of research sites, National Corn and Sorghum Research Center, Experiment II.

	pH	Soil texture	OM	P	K	Ca	Mg
				Before Experiment			
Control	7.1	Clay	M	VH	VH	H	H
				After Experiment			
N Rate							
0 kg ha^{-1}	6.9	Clay	M	H	VH	H	H
25 kg ha^{-1}	7.1	Clay	M	H	VH	H	H
50 kg ha^{-1}	7.4	Clay	M	H	VH	H	H
75 kg ha^{-1}	7.5	Clay	M	H	VH	H	H

§ L = low, M = moderate, H = high, VH = very height.

Appendix C Meterological data at National Corn and Sorghum Research Center, Experiment I.

Month	RH	Temperature (^{o}C)		Total Rainfall
	(%)	Maximum.	Minimum	(mm)
June	77	31.0	23.3	50.0
July	72	31.3	23.7	43.6
August	77	30.6	22.9	151.8
Sept	83	29.4	22.4	363.7

Appendix D Meterological data at National Corn and Sorghum Research Center, Experiment II.

Month	RH	Temperature (^{o}C)		Total Rainfall
	(%)	Maximum.	Minimum	(mm)
November	73	28.0	20.1	9.4
December	65	27.3	17.0	0.0
January	58	28.3	15.4	0.0
February	63	33.2	20.1	47.9

Appendix E Balanced ANOVA for Variate (Mean square) as affected by nitrogen rate and plant population density, Experiment I.

Source of variation	DF	SLW-V5	SLW-R5	TDM-V5	TDM-R5
Replication	2	0.17	0.36	6.9E+04	2.3E+06
Plant density (D)	3	0.67*	0.40*	2.1E+06**	7.1E+07**
Error (a)	6	0.19	0.20	2.4E+04	1.2E+06
Nitrogen (N)	3	4.08**	1.10**	7.5E+04**	3.8E+06**
D x N	9	0.53	0.21	1.4E+04	2.2E+06
Error (b)	24	0.20	0.11	6.3E+03	1.4E+06
Total	47	0.54	0.23	1.6E+05	6.1E+06

* and ** are significant at P<0.05 and P<0.01.

Appendix E (Continued)

Source of variation	DF	100-SW	SY	SP	SO
Replication	2	9.04	1018910	0.47	8.07
Plant density (D)	3	0.41	228857	1.39	0.16
Error (a)	6	1.17	245013	2.50	0.92
Nitrogen (N)	3	7.42**	906365**	5.83*	0.63
D x N	9	0.22	74637	1.84	0.59
Error (b)	24	1.17	100739	1.38	0.41
Total	47	1.67	212830	1.86	0.83

* and ** are significant at P<0.05 and P<0.01.

Appendix F Balanced ANOVA for Variate (Mean square) as affected by nitrogen rate and plant population density, Experiment II.

Source of variation	DF	SLW-V5	SLW-R5	TDM-V5	TDM-R5
Replication	2	0.75	1.55	9.3E+03	3.4E+05
Plant density (D)	3	0.30	0.65*	2.8E+05**	8.5E+06**
Error (a)	6	0.14	0.26	2.0E+04	1.2E+06
Nitrogen (N)	3	0.08	0.64*	4.3E+03	2.9E+06**
D x N	9	0.05	0.08	4.9E+03	4.1E+05
Error (b)	24	0.14	0.18	2.6E+03	1.8E+05
Total	47	0.16	0.29	2.3E+04	1.1E+06

* and ** are significant at P<0.05 and P<0.01.

Appendix F (Continued)

Source of variation	DF	100-SW	SY	SP	SO
Replication	2	1.27	122181	3.39	5.4
Plant density (D)	3	3.06	71636*	2.26	3.7
Error (a)	6	1.58	53789	0.69	2.5
Nitrogen (N)	3	5.39*	821763**	6.32*	4.7
D x N	9	1.07	64009	2.22	1.5
Error (b)	24	1.61	39060	1.74	1.8
Total	47	1.82	101294	2.09	2.3

* and ** are significant at P<0.05 and P<0.01.

Appendix G Balanced ANOVA for Variate (Mean square) as affected by nitrate levels on three soybean cultivars, Experiment III.

Source of variation	DF	S/R-V5	S/R-R5	TDM-V5	TDM-R5
Replication	2	0.59	1.34	0.56	307
Nitrogen (N)	2	1.85*	9.87*	0.82	3858**
Error (a)	4	2.10	9.98	0.68	372.9
Cultivar (V)	2	0.80	1.81	2.02**	324.5
N x V	4	2.33	1.20	0.31	158.2
Error (b)	12	0.39	1.93	0.25	190.3
Total	26	1.11	3.61	0.53	514.9

* and ** are significant at P<0.05 and P<0.01.

Appendix G (Continued)

Source of variation	DF	Chlro-V5	Chlro-R5	LC-R5	LN-R5
Replication	2	4.55	41.24	0.12	0.03
Nitrogen (N)	2	3.94	477.14**	3.05*	4.76**
Error (a)	4	1.25	3.20	0.46	0.13
Cultivar (V)	2	4.87	5.88	2.79*	0.58**
N x V	4	7.88	8.69	0.87	0.38
Error (b)	12	4.21	13.92	0.46	0.05
Total	26	4.38	48.58	0.88	0.51

* and ** are significant at P<0.05 and P<0.01.

Appendix G (Continued)

Source of variation	DF	Branch	Pod	SY	SP
Replication	2	0.1	2308	198.5	2.7
Nitrogen (N)	2	6.8*	21071**	2150*	309.8**
Error (a)	4	1.2	1352.2	158.1	7.0
Cultivar (V)	2	0.4	619.4	336.1*	9.7
N x V	4	1.9	733.8	126.4	10.3
Error (b)	12	1.1	356.7	24.9	8.5
Total	26	1.6	2331.6	261.8	31.4

* and ** are significant at P<0.05 and P<0.01.

Appendix H Hoagland’s stock solution

	Macro	mM	M	MW	g/l	Stock 100x
Stock I	$Ca(NO_3)_2$	2.5	0.0025	236.15	0.59038	59.0375
	KNO_3	2.5	0.0025	101.11	0.25278	25.2775
Stock II	$MgSO_4$	1	0.001	246.48	0.24648	24.6480
	KH_2PO_4	1	0.001	136.09	0.13609	13.6090
	Micro	µM				Stock 1000x
Stock III	H_3BO_3	46.2	0.0000462	61.83	0.00286	2.8565
	$MnCl_2$	9.2	0.0000092	197.90	0.00182	1.8207
	$CuSO_4$	0.38	0.00000038	249.68	0.00009	0.0949
	$ZnSO_4$	2.4	0.0000024	287.54	0.00069	0.6901
	$NaMoO_4$	1.2	0.0000012	241.95	0.00029	0.2903
	Fe(III)EDTA					Stock 100x
Stock IV	$FeSO_4.7H_2O$	90	0.00009	278.02	0.02502	2.5021
	EDTA	90	0.00009	372.24	0.03350	3.3502

§ m*M* = milli-mole, µ*M* = micro-mole, *M* = mole, MW = molecular weight.

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