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EFFECTS OF LIGHT ON ENDOGENOUS SEED ABSCISIC ACID LEVELS AND SEED GROWTH CHARACTERISTICS IN SOYBEAN

A Dissertation Presented

by

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Department of Plant and Soil Sciences

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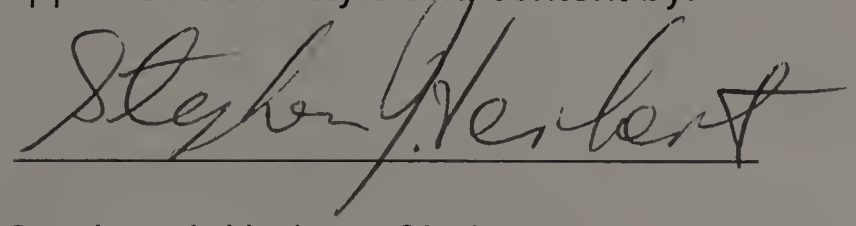

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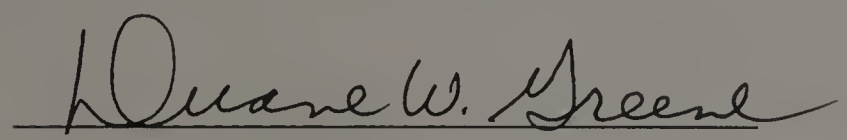

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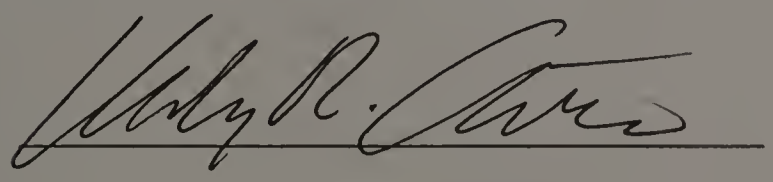

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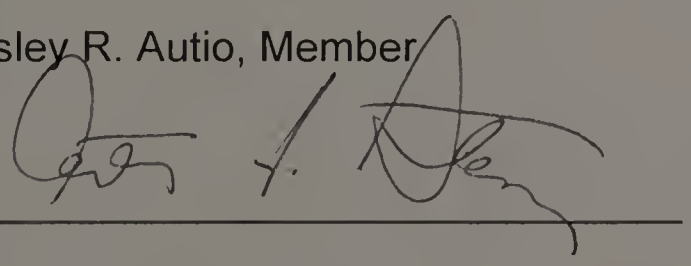

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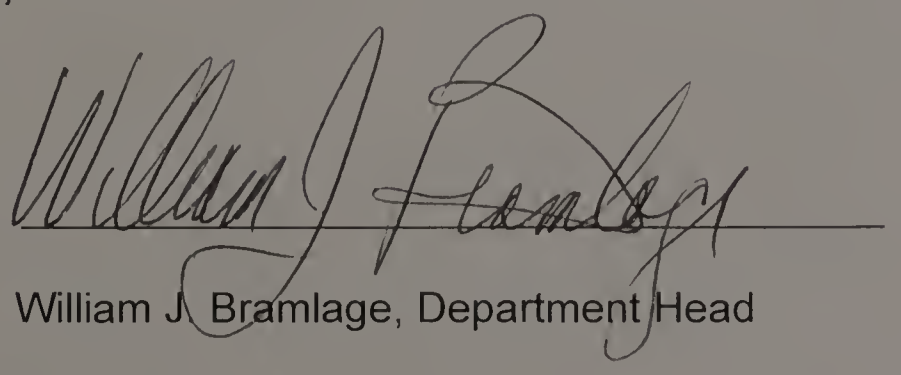

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ABSTRACT

EFFECT OF LIGHT ON ENDOGENOUS SEED ABSCISIC ACID LEVELS AND SEED GROWTH CHARACTERISTICS IN SOYBEAN

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The present study investigated the effects of light variation on the endogenous ABA levels in seed tissues, cotyledon cell characteristics and seed growth rate in soybean (*Glycine max* (L.) Merrill). Yield response of short season soybean Evans of maturity group 0 was studied under light enrichment and reduction treatments. Endogenous ABA levels were determined in seed components and correlated with cotyledon cell characteristics, seed growth rates, seed filling duration and seed size in experiments conducted in 1994-1995.

Optimizing light condition during the early flowering stage (beginning flowering, LE_1) was more efficacious in determining seed yield than at the later growth stage (beginning pod formation, LE_2). The increase in seed yield was largely due to an increase in pod number per plant and per node, although an increase in seed size also partially contributed to increased seed yield in LE_2.

Endogenous abscisic acid levels of seed components was high during the initial stages of seed growth and development and were significantly correlated with the number of cells in the cotyledons and seed growth rates under the light treatments. Plants subjected to shade early in seed development showed lower seed growth rates and a reduction in both final seed weight and cotyledon cell number along with a significant lowering of endogenous ABA levels in the seed components. This trend in seed size variation was consistent across all node positions on the main axis regardless of the light treatments.

Position of a seed in a pod influenced its growth characteristics. Seed growth rate, seed size and cotyledon cell number of the middle and terminal seeds were significantly higher than that of the basal seed. This difference in seed size was consistent across node position, genotypes and irrespective of the number of seeds in pods, thus suggesting that the seeds growth rate is at least partially determined by the genetic constitution of the seed. Thus, within a single genotype, seed size variation within a pod may be influenced more by the rate of seed growth and cotyledon cell number than the filling duration and cell size.

Results indicate that seed growth rate and cotyledon cell number can be influenced by light availability to individual plants, this helps explain variations in seed size within a single genotype that have been observed between years and locations. Reduction in light availability along with lowering of ABA levels during critical cell division period may decrease assimilate availability to the

developing seed, and thus may regulate cotyledon cell number and seed growth rates during the remainder of seed filling period.

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CHAPTER 1

LITERATURE REVIEW

Introduction and Background

Soybean (*Glycine max* (L.) Merrill) is an important crop in the United States as well as in many countries of the world and has been studied for many years with regard to yield. Seeds are the primary sinks for photosynthates during reproductive growth in soybean; thus, an understanding of factors which control the sink activity during reproductive growth is necessary to understand the yield process. Assimilate availability, which is determined in part by source-sink ratio, is one factor that may control the rate of seed growth and final seed weight.

Soybean yield is a function of differential utilization of photosynthates between vegetative and reproductive structures (Shibles and Weber, 1966). Sucrose produced within the mesophyll can be partitioned to either the vacuole for temporary storage or be moved to sieve elements and companion cells where it is loaded for phloem transport. In developing seeds, phloem transport terminates in maternal tissue and sucrose moves apoplastically to the developing zygotic tissue where it is utilized (Thorne, 1985). Concentration gradient of photoassimilates between the source and sink is the major determinant of the rate of transport and partitioning (Giaquinta, 1983).

While the rate of seed growth and final seed size are genetically limited, environmental conditions during seed growth and development can modify

these growth components (Egli et al., 1989). Improved adapted varieties, better cultural methods, increased fertility, and improved machinery are important factors in yield increases per acre (Probst and Judd, 1973). Many environmental factors such as solar radiation, soil fertility, soil aeration, soil and air temperature, carbon dioxide, moisture, weed pressure, insects and pathogens also affect soybean growth and final yield (Litchfield, 1991; Norman, 1978).

Light and Light Interception in Soybean

Solar radiation plays a significant role in determining the yield as it effects both vegetative growth and accumulation of dry matter in developing seeds of crops. Visible light, which makes up about 40-50% of the incident solar radiation, is the fundamental source of energy for photosynthesis and carbohydrate production in plants (Luxmoore et al., 1971). The visible band is made up of that part of the solar spectrum from 400-700 nm and is referred to as photosynthetically active radiation (PAR) and is measured as photosynthetic photon flux density (PPFD) (Gallo and Daughtry, 1986). The PPFD is the number of photons in the PAR wave band that are incident on a unit surface in a unit time (Shibles, 1976). More efficient light interception by soybean canopies contributes to an increased yield (Duncan, 1986; Ikeda, 1992).

Dry matter accumulation is dependent directly on light intensity intercepted by the photosynthetically active plant parts. A linear relationship occurs between photosynthetic active radiation and crop dry matter

accumulation (when moisture and nutrients are not limiting) and the slope of the linear relationship (i.e. efficiency of conversion of solar radiation into plant material) varies with crop species and phase of crop development (Tollenaar and Bruulsema, 1988). Radiation interception by a crop is thought to limit productivity when other environmental factors are not limiting (Loomis and Williams, 1963). Seasonal and daily fluctuation of solar radiation in the field can also contribute to the variation in source supply to the sink.

The effect of canopy architecture (orientation of leaves) on radiation penetration and distribution in canopy is also thought to be a major determinant of photosynthetic efficiency and growth (Ottman and Welch, 1989). In soybean crop canopy there is an uneven distribution of light on the surface of leaves. Soybean crop has a large leaf area and short plant height, thus having a more closed canopy than crops like corn. Sakamoto and Shaw (1967) reported that 90% of incoming light was intercepted in the top 10% of the canopy. Herbert and Litchfield (1984) reported that improvements in the canopy architecture, developed by narrowing row widths resulted in higher net assimilation rates indicating a more optimum canopy display of leaves was achieved which resulted in greater seed yields than in wide rows.

A number of studies on soybean indicate that although most of the light interception occurs at the top and the periphery of the canopy, most yield occurred in the lower central part of the plant (Herbert and Litchfield, 1982). The leaves in the lower canopy contributed little photosynthates to the plant yield (Shibles and Weber, 1965; Johnston et al., 1969). This might suggest that

long distance assimilate translocation exists in soybean plants, through which assimilates are transported from upper and periphery nodes to the middle and lower nodes. However, Stephenson and Wilson (1977) reported most of the photoassimilates produced by leaves and partitioned to seeds during pod fill remained in pods and seeds at the node of attachment. Wilcott et al. (1984) determined that leaves attached in the lower middle portion of the plant are displayed due to long petioles, much higher in the canopy where light is more available.

Artificial shading (light reduction) decreased soybean yields by up to 30-40% (Egli et al., 1980) and increased lodging (Allen, 1975). Reducing the supply of photosynthates through shading or defoliation caused reduction in the carbohydrate levels in the leaves and other plant parts (Thorne and Koller, 1974). Light enrichment increased plant yields due to an increase in available light for each plant, as more light penetrated into the lower canopy parts (Johnston et al., 1969). More information on the factors which influence the rate and duration of seed growth is needed to understand the yield production process.

Johnston et al. (1969) used wide-spectrum fluorescent lamps to increase light on soybean canopies. They showed that adding light resulted in an increase in pod number per plant and seeds per pod but there was a reduction in the seed size. Wahua and Miller (1978) applied shade treatments to soybean plants which reduced light by 20% - 93% as compared to ambient light. Their results showed a highly negative correlation between the amount of

shading and grain yields, pod number per plant, and percent leaf nitrogen. These studies showed that solar radiation greatly affects soybean yield.

Schou et al. (1978) imposed light enrichment and reduction to soybean plants at different stages of development. One method involved installation of reflectors at 45^0 angles to the sample row and installation of black boards of similar dimension in the same manner. Light enrichment treatments showed an increased yield of 6%-57% more than the controls. Plants that were light enriched during the period of late flowering to early pod formation stage had 48% more pods, and 57% more seed yields than the controls. Light reduction of about 63% was achieved by using shade cloth. Shade treatment imposed during the same developmental stages showed a reduction in both pod number and seed yields (16% and 29% respectively) than the control treatment. The authors postulated that the soybean plants filled more pods and more seeds due to the rich light condition.

Yield of a grain crop is a function of rate and duration of dry-matter accumulation in the seed. Like most C3 plants, soybean has a low photosynthetic efficiency, which is defined as the fraction of energy fixed biochemically by the plant to that energy incident on the plant as measured in the PAR band, or in the total solar spectrum. Many researchers have conducted experiments on the effects of increased light penetration into and shading of soybean crop canopies. Improvement in light utilization by the soybean canopy through planting patterns such as plant density, row width, and intercropping are being thoroughly investigated.

Another important aspect of light in soybean production is the photoperiod that resulted in classification of soybean maturity groups in the United States. There are 11 zones in North America of best adaptation for soybean cultivars corresponding to Maturity Group 00 through Maturity Group IX. Soybean cultivars that are grown in Massachusetts belong to the Maturity Group II. However, Group 0 and Group I are usually planted in this area to ensure an earlier harvest. The placing of soybeans into maturity groups plays an important role in increasing soybean yields. Snyder and Kwon (1987) found that yield of soybean could be reduced if a maturity group which was adapted for the northern latitudes was planted further south as the plant would flower and set pods before it reached its full vegetative growth. Similarly, a plant which is adapted to the southern region, if grown in the north would have its flowering postponed and could easily encounter early frost before the seeds matured. Photoperiod can strongly influence the morphology of soybean plant by changing the time of flowering and maturity, which can result in differences in plant height, pod weight, leaf area, lodging, dry matter production and final grain yield (Norman, 1978).

Seed Growth Rate and Duration of Seed Filling

Along with the changes in the environment such as irradiance, temperatures and internal factors like the position of the kernel in an ear of corn and removal of the leaves, kernels or pods all affect the final yield in grain crops. Higher temperatures (30/25˚C) have been found to reduce the duration

of seed growth as they hasten leaf senescence (Egli and Wardlaw, 1980). On the other hand, cool temperatures (10/15°C) increased the duration of the lag phase (endosperm cell division) and increased the effective grain filling period in corn and resulted in a reduction of the kernel growth rate and final seed mass (Jones et al., 1981). Seed growth rates have also been found to be influenced by temperatures during flowering and pod set which suggested that the seed growth rate is at least partially determined by events occurring early in seed development.

In soybean both the rate and the duration of seed filling are important parameters in the yield production. Egli et al. (1981) reported that a comparison of seed growth characteristics of a few soybean genotypes have shown that seed size is more closely correlated with the growth rate than with the filling duration, both of which are under the genetic control. Guldan and Brun (1985) found little differences in seed filling duration in three soybean plant introductions which showed different seed growth rates and sizes. Swank et al. (1987) reported significant differences in seed size among soybean genotypes with no differences in growth rates, indicating that differences in seed size were associated with a variation in the duration of the filling period.

Cotyledon Cell Characteristics

Final seed size has been positively associated with the rate of dry matter accumulation and number of cells in cotyledon or endosperm across a number of plant species. During endosperm cell division, a potential sink capacity or

sink strength is established and yield may be determined by number or size (or both) of endosperm cells or starch granules, which may ultimately regulate the metabolic activity in endosperm (Reddy and Daynard, 1983). The number of cells in wheat endosperm is presumed to be a major determinant of the capacity of the grain to accumulate dry matter (Jenner, 1979). A positive correlation has been reported between the number of cells in cotyledon and final dry matter of the mature pea seed (Davies, 1975). Greater number of cells in sugarbeet tap root resulted in a greater assimilate accumulation (Doney et al., 1981). This is assumed to occur by an increase in cell surface area leading to an enhanced capacity for carbohydrate uptake.

In soybean, Egli et al. (1980) reported genetic differences in the seed growth rates of soybean were related to the number of cells in cotyledons. Guldan and Brun (1985) found a positive correlation between soybean cotyledon cell number and the ability of the seed to accumulate dry matter. They reported cell division in soybean to terminate approximately 8-10 days after anthesis and subsequent growth to occur by cell enlargement. However, within the same soybean genotype, it has been reported that seed size may be influenced more by cotyledon cell size than by number (Hirshfield et al., 1992) as significant increases in the final yield of soybean with fertilizer treatment were observed which were due to an increased size of the cotyledon cells rather than an increase in cell number.

The dry mass of the seed was found to increase at an exponential rate during the short initial phase of growth, followed by a period when the rate of

accumulation of dry mass is relatively constant and a period of decreasing growth rate until seed reached maximum dry weight at physiological maturity (Fraser et al., 1982). This early stage is the period when the cell division occurs in the endosperm (Briarty et al., 1979) and is suggested to influence final grain weight by their effect on number of endosperm cells formed (Brocklehurst et al., 1978). The magnitude of the responses to environmental or physiological conditions was found to be greater when the treatment was given during the first 10-15 days after anthesis than during the later stages of seed development (Fischer and Laing, 1976).

Studies have shown that unfavorable temperatures during the endosperm cell division in wheat reduced endosperm sink capacity by reducing cell size with little or no effect on cell number (Radley, 1978). Thompson et al. (1977) reported that *in vitro* cotyledon growth rate could be sensitive to reduced levels of sucrose or nitrogen containing compounds in the phloem (Egli et al., 1980) thereby explaining some of the environmental effects on the seed size.

Role of Endogenous ABA in Plants

A close examination of the various components involved in the process of partitioning indicate that endogenous plant hormones may serve as natural regulators of many of the specific rate limiting processes (Brenner and Cheikh, 1995). Plant growth regulators such as cytokinins, auxins, gibberellins and abscisic acid are found in relatively high concentrations in the extracts from

seeds of different developmental stages and are known to be involved in determining both the sink size and its capacity.

Abscisic acid (ABA) is a sesquiterpene which is derived either from mevalonic acid or xanthophyll (Walton and Li, 1995). It was earlier considered only as an inhibitory substance (Walton, 1980). Dry dormant seeds are usually found to contain a higher concentration of endogenous ABA than the nondormant ones. ABA was very effective in causing stomatal closure and its accumulation in stressed leaves played an important role in the reduction of water loss by transpiration (Horton, 1971). ABA is known to be involved in senescence, and through its promotion of senescence, it indirectly increases ethylene production which stimulates abscission (Taiz and Zeiger, 1991). ABA is reported to reduce photosynthate production directly (Raschke and Hedrich, 1985) by a reduction in ribulose- 1,5- bisphosphate carboxylase activity (Fisher et al., 1986). Increase of ABA in leaves caused stomatal closure resulting in a decline in photosynthesis due to low intracellular CO_2 levels.

Abscisic acid (ABA) is recognized as a naturally occurring plant hormone of major importance in the regulation of many aspects of plant growth and development (Milborrow, 1974; Walton, 1980). Developing fruits have been shown to contain in addition to cytokinins and auxins, a high concentration of ABA during the grain filling period (King, 1976). Research shows ABA to be actively involved in the regulation of transport and storage of assimilates during the development of grains in a number of plants. ABA levels have been found to rise sharply and then fall during the development of seeds of wheat (King,

1976), soybean (Quebedeaux et al., 1976), pea (Eeuwens and Schwabe, 1975), bean (Hsu, 1979), and barley (Goldbach and Goldbach, 1977).

Translocation of photoassimilates to the fruits and storage organs and synthesis of storage materials all affect the development of seed. While many studies have examined this process, it is not fully understood which processes control and affect seed growth. One of the problems encountered in studying the ABA biosynthetic pathways is in the poor incorporation of its radioactive precursors into ABA (Walton and Li, 1995). The exact role of ABA in seed growth and development is difficult to ascertain, as it is difficult to regulate endogenous ABA levels in developing seeds. ABA can either be synthesized within the seed or be translocated from other parts of the plant to the seed. There is evidence that the mature fully expanded leaves are a major source of ABA production and the hormone is exported to other parts of the plant via the xylem and the phloem (Wolf et al., 1990).

Phloem unloading is a process by which translocated sugars exit from the sieve elements of the sink. This transport of sugars is energy dependent, as sugars accumulate in high concentration and are moved against a concentration gradient. Tietz et al. (1981) suggested the possible involvement of ABA in the regulation of assimilate transport from leaves to the grains by influencing the unloading of sieve tubes in the ears of barley. Clifford et al. (1986) gave a direct evidence of the involvement of ABA in the unloading of phloem-derived photosynthate into the apoplast of the *Phaseolus* seed coat. Ross (1987) reported an increased phloem unloading into the seed coat of pea

in the presence of 10^{-5} mol m^{-3} ABA, but at a higher concentration, the response was less clear. Experiments with wheat have shown that ABA when injected into the grain markedly increased the movement of $^{14}CO_2$ labeled photosynthetic assimilate towards the ear (Dewdney and McWha, 1979), thereby explaining the involvement of ABA in phloem unloading.

In soybean, it has been reported that the embryos require ABA for continued development and for the accumulation of storage proteins (Eisenberg and Mascarenhas, 1985). Schussler (1984) found a high concentration of ABA in large-seeded genotypes of soybean as compared to the small and the medium sized seeds. He proposed that ABA may be involved in the stimulation of rapid unloading of sucrose into the seed coat of soybean and ABA in cotyledons may enhance sucrose uptake by the cotyledons. Rapid increases in the fresh and dry weights of the soybean seeds were found to be correlated with a peak in the rate of ABA accumulation in the developing seeds (Quebedeaux et al., 1976). Similarly, a decrease in the rate of dry-weight accumulation was associated with a sharp decline in ABA concentration.

However, in contrast to the above results, there is evidence that ABA lowered the assimilate-transport intensity as ions and assimilates were found to move away from the site of ABA application (Mullins, 1970). A decline in the RNA synthesis in bean was observed with an accumulation of endogenous ABA (Hsu, 1979). Inhibition of sucrose uptake by castor bean cotyledons when incubated in the presence of ABA was reported by Vreugdenhill (1983). Similarly, Porter (1981) found an inhibitory effect of ABA on the translocation of

assimilates to lupin inflorescence. There have also been reports which show no effect of exogenous ABA applications on storage protein accumulation in isolated pea embryos (Davies and Bedford, 1982).

The traditional techniques for studying the influence of growth regulators on physiological processes are done either by the application of the compound under study or by the inhibition of its synthesis or activity by chemical means. Exogenous ABA applications have been found to influence assimilate accumulation in legume seeds, however, the results are often found to be variable. This can be explained in part by the changes in the sensitivity of the tissue to variability in the level of the hormone concentration. Tietz (1981) found that exogenous ABA applications at the time when the endogenous ABA levels were low, caused a stimulation of ^{14}C- assimilate transport from the flag leaf to the developing barley grain and increased the final weight per grain. However, if ABA was applied when the endogenous levels were high, exogenous ABA was found to decrease ^{14}C transport and resulted in a reduction or had no effect on the grain weight. The main disadvantage of these techniques is that the exogenous application of the growth regulator could lead to its availability at an inappropriate place, time or concentration and the inhibition of hormone synthesis or activity could lead to undesirable side effects and cause injury to the tissues (deBruijn and Vreugdenhil, 1992).

Application of immunoassays for the quantification of plant hormones has been of much interest in recent years and is now a recommended method for the quantification of ABA and related compounds (Hirai, 1986).

Immunoassays are based on the ability of the animals to produce proteins (antibodies) which recognize and bind to specific compounds (antigens) foreign to the animals. Quantification of hormones from small amounts of plant tissue can be achieved because of the specificity and sensitivity of antibodies. The assay principle uses the comparative antibody binding method to measure concentration of hormones in the plant extract.

While growth rate and final seed size in soybean are genetically limited, the environmental conditions during seed development can modify these seed growth components (Egli et al., 1985). Short day photoperiod can increase the partitioning of assimilates to filling seeds of soybean, resulting in higher seed growth rates as reported by Morandi et al., (1990). They found an earlier accumulation of ABA and sucrose in short day plants and suggested that ABA may have increased assimilate availability during the critical cell-division period, thus regulating cotyledon cell number and subsequent seed growth rate for the seed-filling period. Ackerson (1980) found that during the phase of early embryogenesis, higher concentration of endogenous ABA levels were correlated with greater embryo growth rates in soybean. From these data it was suggested that cell division during embryo development was stimulated by ABA.

Increase in the size of seed has been positively correlated with the plant performance in the field in a number of crop species (Beveridge and Wilsie, 1959; Black, 1957). In soybean, size of the seed has been positively correlated with the yield potential (Fehr and Probst, 1971). Burris et al. (1973) found the

larger seed sizes of soybean variety to yield significantly more than the small sized seeds when grown at uniform populations.

An indeterminate soybean plant usually blooms at first at the unifoliate or second main axis node. Flowering progresses upwards, and many new leaves and branches in leaf axils (especially at low plant densities) are developed concurrently with the progression of flowering. Pods are well formed near the base of the plant before the last flower appears at the uppermost nodes. The upper-node-position seeds thus have a potentially shorter seed filling duration as compared to seeds in pods at lower nodes. At maturity, however, Herbert and Litchfield (1982) reported all seeds reached the same size irrespective of their position on the plant. However, within both two and three seeded pods, the basal seed is the first to develop and is the closest to the vascular connection, but it lags behind in dry matter accumulation as compared to the middle and terminal seed. At maturity, it is smaller in size (Herbert and Zhi-yi, 1984). Seed number within a pod varies from 1 to 4 seeds, usually averaging between 2 and 3 seeds. The effect of differences in seed number in pods on seed size is not well documented. The present study investigated the effects light variation on the endogenous ABA levels in seed tissues, cotyledon cell characteristics and seed growth rates in soybean.

Objectives

The main objectives of this research were (a) to determine the effects of light reduction and light enrichment on the growth pattern of soybean plants,

and on seed development, (b) to determine if the changes in the seed size of soybean are correlated with changes in ABA levels in the seed coat and cotyledons, and (c) to determine if changes in seed size caused by light reduction and light enrichment are a consequence of an increased cotyledon cell number and/or cell weight and if the differences in seed number per pod influenced seed size, seed growth rates, filling duration and cotyledon cell characteristics in soybean.

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CHAPTER 2

EFFECT OF LIGHT ENRICHMENT AND SHADING ON SOYBEAN YIELD AND SEED YIELD COMPONENTS

Abstract

Field experiments were conducted to determine the nodal distribution of seed yield components in short season soybean grown under different light conditions. Evans, a maturity group 0 soybean cultivar was planted in late May 1994 & 1995 at a density of 83 plants m^{-2} under light enrichment and shading treatments into a fine sandy loam soil (Typic Udifluvents). Seed yield was enhanced by increasing light availability to individual plants. Increasing light at the early reproductive stage (beginning flowering) was more effective in determining the final seed yield than at the beginning of pod development. The increase in seed yield was contributed largely by an increase in pod number per plant and per node, although an increase in seed size also partially contributed to increased seed yield in LE_2. Compared to controls, an increase in the size of seed by 14% and 21% was achieved when light enrichment was imposed during beginning of flowering and pod formation stage respectively (results averaged over both the years). Seed number per pod and size of the seed across main axis node positions on a plant were constant regardless of the light variation, thus showing it to be under the genetic control of the plant. Node positions 5 to 9 had the greatest pod number and percentage of seed yield as compared to other nodes in all treatments. Harvest index was higher

for early light enrichment as compared to other treatments, an increase in the proportion of economic yield along with an increase in total dry matter accumulation.

Introduction

Rate of dry matter accumulation is dependent on the area of assimilatory organs, mainly leaves (Allison, 1969). The extent of light interception and its distribution through the profile of the plant community has a major role in determining crop productivity (Williams et al., 1968). Percent light interception and rate of dry matter is found to increase with leaf area development (Scarsbrook and Doss, 1973; Bullock et al., 1988). Greater seed yield in narrow row spacing as compared to wide-rows in soybean has been frequently attributed to greater light interception by the crop canopy (Tanner and Hume, 1978).

In soybean much of the radiant light (approximately 90%) is intercepted by the top and periphery of canopy, with a decrease in available energy downwards within the canopy (Sakamoto and Shaw, 1967). Leaves at the canopy surface may become light saturated while leaves lower in the canopy are at suboptimal light levels. As plants grow, light penetration into the canopy is inhibited by increased leaf area and interaction between plants when canopy closure occurs. Thus, many lower leaves may not receive adequate radiation levels to carry on high rates of photosynthesis. Lodging can further reduce light penetration into the crop canopy. It was shown though by Wilcott et al. (1984)

that leaves attached in the lower and middle portion of the plant are displayed due to long petioles, much higher in the canopy where light is more available.

In addition to leaves as the major source of photosynthates in soybean plants, pod walls, stems, and petioles could partially serve as sources of stored carbohydrate for developing seeds (Streeter and Jeffers, 1979; Stephenson and Wilson, 1977). Seed yield in indeterminate soybeans occurs primarily from pods produced on lower central nodes of soybean plant and less from upper nodes (Dominguez and Hume, 1978; Herbert and Litchfield, 1982). In indeterminate soybean plant, almost every node provides pod setting and seed development. The greatest amounts of photoassimilates produced by leaves remained in pods at the node of attachment (Stephenson and Wilson, 1977).

Among the yield components, pod number per plant is the most responsive to changes in the environment (Dominguez and Hume, 1978; Hoggard et al. 1978, Peet and Kramer, 1980; Marvel et al., 1993). Light enrichment during flowering and early pod formation could lead to increased pods per plant at final maturity (Schou et al., 1978 and Johnston et al.,1968).

Seed number per pod is reported to be more stable than pods per plant changing little with widely varying densities and row spacings (Herbert and Litchfield, 1982). However, there are reports that seeds per pod were influenced by changes in row width and plant density (Paudey and Torrie, 1973) and in light interception (Kokubun and Watanabe, 1981). According to Herbert and Litchfield (1982) variation in seed number per pod was most evident at extreme upper and lower node positions because fewer pods at the

extreme node positions contained 1 to 4 seeds which led to a larger deviations from average seed number per pod of central nodes.

Seed size, measured as average dry weight per seed, also is less variable than pod number per plant but is more responsive to environmental changes than seed number per pod. Herbert and Litchfield (1982) reported changes in average seed size accounted for significant differences in seed yield between years.

This present study was conducted to investigate the different effects of light availability to the soybean canopy on final yield and yield components and to determine if light availability might be a key factor influencing yield component variation between years. Yield was analyzed by determining yield components on a node by node basis.

Materials and Methods

Cultural practices

Experiments were conducted in 1994 and 1995 in the Connecticut River Valley at University of Massachusetts Agricultural Experimental Station Farm in South Deerfield, Massachusetts. The soil type is a Hadley fine sandy loam (Typic Udifluvent, coarse-silty, mixed, nonacid, mesic). The experimental site tested high for P, thus it only received an application of muriate of potash at a rate of 306.45 Kg Kha^{-1} prior to cultivation in 1994. Tillage was mold-board plowing and disking prior to planting. Irrigation was not needed in either years.

Evans, an indeterminate, Group 0 soybean, with approximately 92% seed germination was inoculated with commercial powdered-peat inoculant before planting. Seeds were machine planted on May 24, 1994 and May 23, 1995. The experimental design was a complete randomized block design with four replications and each plot size was eight rows wide by 12 m long. Weed control in 1994 consisted of a post emergence mixture of 2.06 liter ha^{-1} poast [2-(1-(ethoxyimino)butyl)-5(2-(ethylthio) propyl)-3-hydroxy-2-cyclohexene] and 2.75 liter ha^{-1} crop oil. In 1995, weed control was done by pre-emergence applications of 1.7 kg ha^{-1} active ingredient (a.1) alachlor [2-chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide] and 0.85 kg ha^{-1} a.i. linuron [3-(3,4-dichlorophenyl)-1-methoxy-1 methylurea], plus hand weeding during the early vegetative growth stage of soybean in both years.

Light Enrichment and Shading Treatments

Light enrichment entailed increasing solar radiation available to the center sample row of each plot at beginning of flowering (LE_1) and pod formation stage, R-stage (LE_2) respectively. This was achieved by installing a 90 cm tall wire mesh fence 25 cm from each side of the sample row, sloping at approximately 45^0 angle away from it. These fences were inspected periodically, and the plants held back by the fence were rearranged to avoid undue crowding to allow near normal growth of these fenced border row plants. Thus, plants from the neighboring rows were prevented from encroaching into the center sample row allowing greater light penetration into the canopy of the

center sample row. This method was chosen to allow light penetrated deeper into the canopy without disturbing the root system of plants in border rows. Once put in place, these fences remained in position until soybean maturity. Shade (S_2) was provided by using black polypropylene fabric installed 0.5 m above the soybean canopy at the R-stage (LE_2), and shade cloth once installed remained in place until seed maturity. Shade cloths were attached to a vertically movable wire trellis attached to wooden posts. A control treatment (LE_0) received no light enrichment or shade.

Interception of Photosynthetically Active Radiation

Light measurements were taken above, within, and below the canopy, of the available photosynthetically active radiation (measured in microeinsteins per m^2 per s) for all the treatments at the beginning of pod development in 1994 and 1995. Measurements were obtained with a Li-cor line quantum sensor (LI-1915B) and integrating quantum/radiometer/photometer (LI-188B), at 1100 to 1350 hours on eastern daylight time on days when clouds caused no interference. Inter-row light readings were taken with the light sensor placed across the inter-row space. All readings were integrated over one meter and ten seconds.

Seed Yield and Yield Components

Final yield for LE_0 and S_2 was determined by harvesting plants in 4 rows with a length of 3m. For light enrichment treatments, 3m of the central row

between the fences was harvested. For yield components analysis, 15 plants in each plot were harvested from a random starting point at physiological maturity and data were recorded for the whole plant as well as for each node position on the main axis. Data recorded were pod number, seed number, stem dry weight, pod dry weight and seed dry weight. Harvested plants were hand threshed and oven dried at approximately 80°C to a constant weight. Harvest index was the ratio of seed yield to total above ground biomass at harvest.

Results and Discussion

Interception of PAR

Light intensity at the top of canopy at beginning of pod formation stage was 13.06 and 7.34 $uEs^{-1}m^{-2}$ for the unshaded and shaded treatments respectively (Fig. 2.1 - results averaged over 1994 and 1995). At the base of canopy it was 0.19, 3.37 and 0.03 $uEs^{-1}m^{-2}$ for LE_0, LE_2 and S_2 respectively (Fig. 2.2). Shading resulted in a 52% light reduction compared to the control. Thus 98.6% and 74.3% of incoming solar radiation was intercepted by the soybean crop canopy for LE_0 and LE_2 respectively. The leaves at the base of canopy in LE_2 were receiving more than 25% of the available light.

Yield and Yield Components

The final yield and yield components for Evans in 1994 &1995 are shown in Tables 2.1& 2.2. Results from these experiments support the

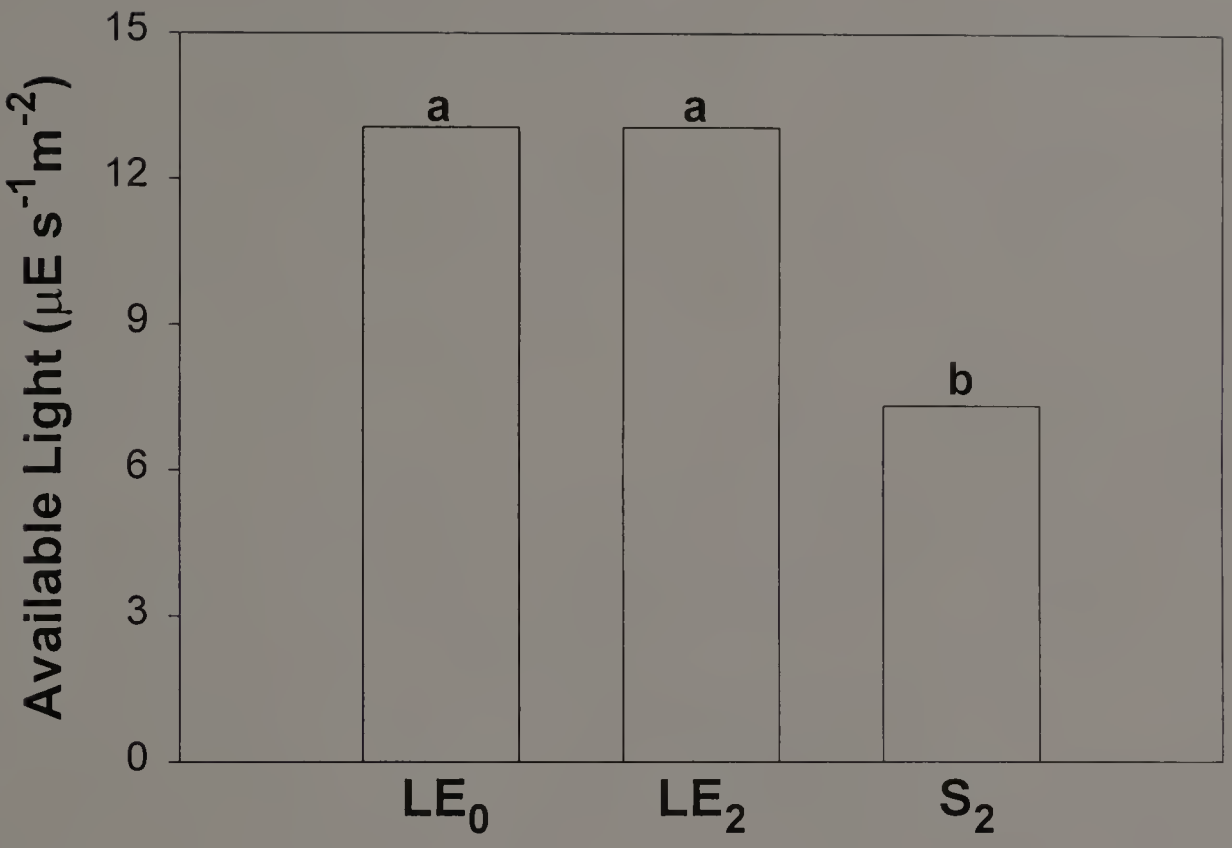

Figure 2.1 Light available at the top of the canopy in light treatments.

LE_0: Control; LE_2; Light enrichment; S_2: Shade

- Results averaged over 1994 and 1995
- Means followed by the same letter are not significantly different at the 5% level of probability using Duncan's Multiple Range Test.

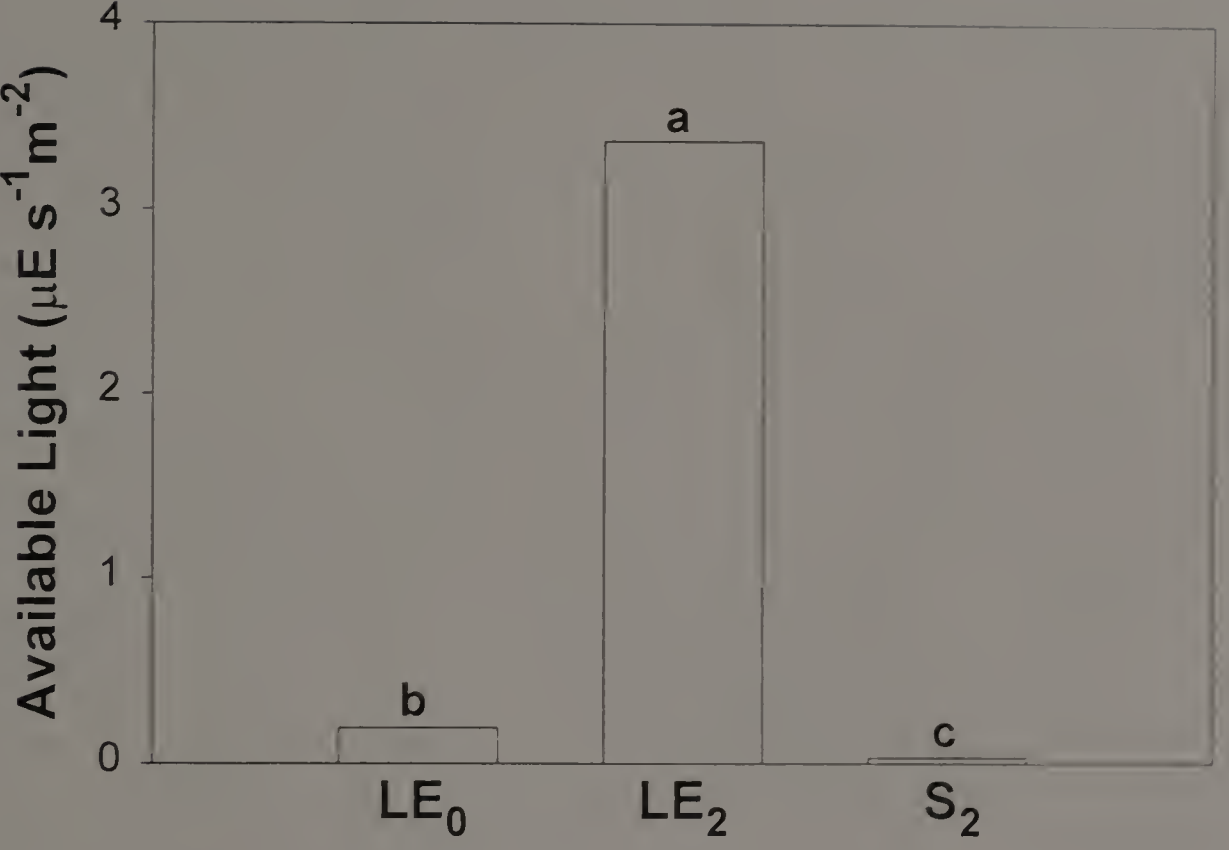

Figure 2.2 Light available at the base of the canopy in light treatments.

LE_0: Control; LE_2; Light enrichment; S_2: Shade

- Results averaged over 1994 and 1995
- Means followed by the same letter are not significantly different at the 5% level of probability using Duncan's Multiple Range Test.

conclusion that an improvement in light environment early in the season during reproductive growth stage (LE_1) was more effective in increasing yield (236%) compared to control, while light enrichment initiated at late flowering (LE_2) resulted in a 72% increase in seed yield compared to control (results averaged over both the years). Seed yield increases in LE_2 significantly differed from the control in 1995 only and no differences were observed in 1994. Litchfield (1991) reported a 124% increase in seed yield in similar treatment of light enrichment, imposed on soybean plants 10 days prior to the first flower. Thus, the critical time when an improved light interception resulted in an increased soybean yield was the beginning of reproductive growth stage, when the first flower appeared.

The increase in number of pods per plant at maturity was the yield component most responsible for an increase in yield over the control (Tables 2.1 and 2.2). In 1994 and 1995 respectively, there was a 163% and 125% increase in pod number per plant in LE_1 which accounted for most of the increase in seed yield per plant compared to LE_0, while there was only a 19% and 25% increase in pod number per plant for LE_2 which did not differ significantly from the control. Jiang and Egli (1993) found a reduction in pod number resulting from shade treatment applied during the flowering stage was due to both increased flower and pod abscission and a result of fewer flowers developing per plant. The increase in seed yield in LE_1 treatment was probably from an increase in flowering, and from a reduction in flower abscission. However, an increase in pods per plant from light enrichment imposed when

Table 2.1 Final yield, yield components and harvest index for soybean under light treatments in 1994.

Treatments	Seed Yield (m^{-1})	Pods $Plant^{-1}$	Seeds Pod^{-1}	Seed Size (mg dry wt $seed^{-1}$)	Harvest Index
LE_0	87.2 b	13.5 b	2.03 b	170.0 b	0.61 b
LE_1	306.3 a	35.6 a	2.32 a	199.1a	0.68 a
LE_2	118.6 b	16.0 b	2.18 a	202.9 a	0.61 b
S_2	62.8 b	11.5 b	1.94 b	159.2 b	0.59 b

- Data points are the means of seeds averaged over 4 replications.
- LE_0, LE_1, LE_2 and S_2 represent control, light enrichment at the beginning of flowering, light enrichment at the beginning of pod development and shade at beginning of pod development, respectively.
- Means within columns followed by the same letter are not significantly different at the 5% level of probability using Duncan's Multiple Range Test.

Table 2.2 Final yield, yield components and harvest index for soybean under light treatments in 1995.

Treatments	Seed Yield (m^{-1})	Pods $Plant^{-1}$	Seeds Pod^{-1}	Seed Size (mg dry wt $seed^{-1}$)	Harvest Index
LE_0	72.07 c	13.32 b	2.10 b	162.1c	0.50 b
LE_1	228.93 a	29.92 a	2.20 a	179.3 b	0.64 a
LE_2	155.45 b	16.58 b	2.14 b	199.3 a	0.59 ab
S_2	65.35 c	6.60 c	1.72 c	160.5 c	0.41 c

- Data points are the means of seeds averaged over 4 replications.
- LE_0, LE_1, LE_2 and S_2 represent control, light enrichment at the beginning of flowering, light enrichment at the beginning of pod development and shade at beginning of pod development, respectively.
- Means within columns followed by the same letter are not significantly different at the 5% level of probability using Duncan's Multiple Range Test.

the flowering was almost complete (LE_2), was mainly as a result of increased pod retention (Litchfield, 1991).

Seed number per pod (Tables 2.1 and 2.2) was increased for LE_1 compared to LE_0 and S_2. However, this increase was small in comparison to the increase in pod number per plant. This slight increase in seed number per pod in LE_1 indicates than an improvement in light availability to plants at early reproductive growth stage led to sufficient source supply to the newly formed zygotes, embryos or young seeds that otherwise in poor light conditions might fail to develop during meiosis.

Seed size (weight per seed) exhibited significant increases for both LE_1 and LE_2 over control (Tables 2.1 and 2.2). Plants subjected to LE_2 showed a significant increase in the final seed size as compared to LE_1 in 1995 only. This is because an increase in light interception when most of the flowers had set (LE_2), resulted in no further increase in pods per plant and seeds per pod, whereas significant increases in pods per plant and seeds per pod occurred with earlier light enrichment (LE_1). The sink size capacity (number of pods or seeds) in LE_2 was less than in LE_1.

Harvest index for LE_1 was increased significantly by 11% and 28% above the harvest index of the control in 1994 and 1995 respectively (Table 2.1). The yield in soybean can be enhanced either by increasing the total dry matter accumulation or by increasing the proportion of economic yield (harvest index) or both (Gardner et al., 1985). Here, the yield increase which resulted from an improvement in solar radiation available to soybean plants, especially

in early reproductive growth stage (LE_1), was a result of an increase in total dry matter and also to the higher harvest index, which indicates an improvement in partitioning of assimilates to reproductive yield.

The distribution of pods across main axis node positions of soybean plants are shown in Fig. 2.3. Significant differences in pod number per plant due to light treatments suggests that solar radiation became important in determining pod setting early in the reproductive period. The majority of pods were borne on central region of plant. Previous studies (Herbert and Litchfield, 1982; Egli, 1988; Litchfield, 1991; Jiang and Egli, 1993) have suggested the nodes in central region have the highest yield potential in soybean plants. The increase in light availability in LE_1 in 1994 led to an increase in pods across all nodes on the main axis of the plant, more than in LE_2, showing soybean plants are most responsive to an increase in solar radiation at an early reproductive stage. However, in 1995 the differences in pod numbers across the nodes on main axis under different light treatments were less clear.

Seed number per pod was relatively constant across node positions, except at the upper extreme node positions (Fig. 2.4). The characteristics of relatively constant seeds per pod across main axis node positions suggested that pod anatomical organization was controlled genetically, rather than being under the environmental influence. Increased solar radiation did not largely improve the mean seed number in pods across stem node positions. The significant increase in seeds per pod in LE_1 compared to LE_0 in both years indicates that improved light environment at early reproductive growth stage

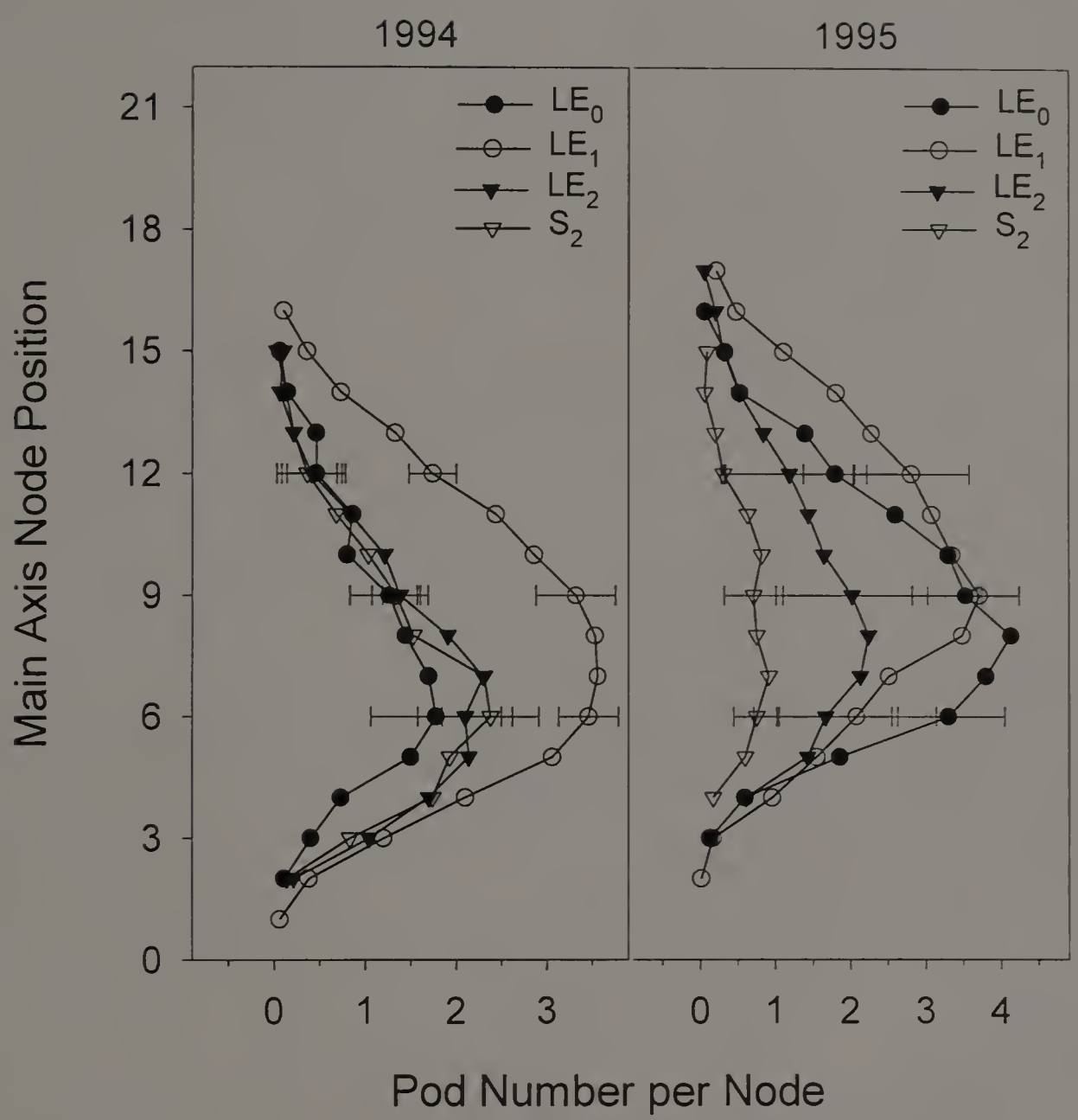

Figure 2.3 Pod number per main axis node position in response to light treatments.

LE_0: Control; LE_1: Light enrichment beginning flowering; LE_2; Light enrichment at pod development; S_2: Shade at pod development.

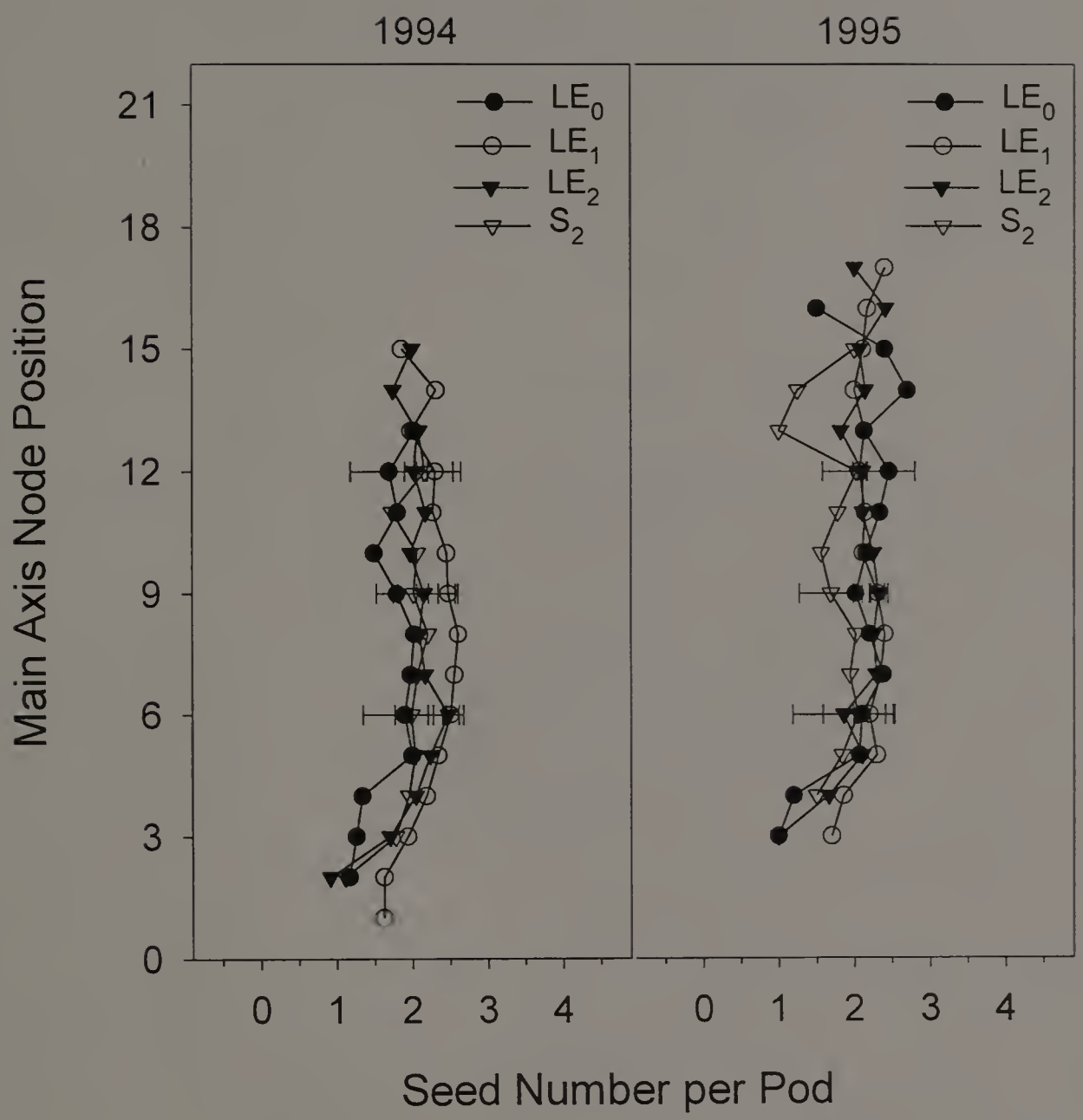

Figure 2.4 Seed number per pod across main axis node position in response to light treatments.

LE_0: Control; LE_1: Light enrichment beginning flowering; LE_2; Light enrichment at pod development; S_2: Shade at pod development.

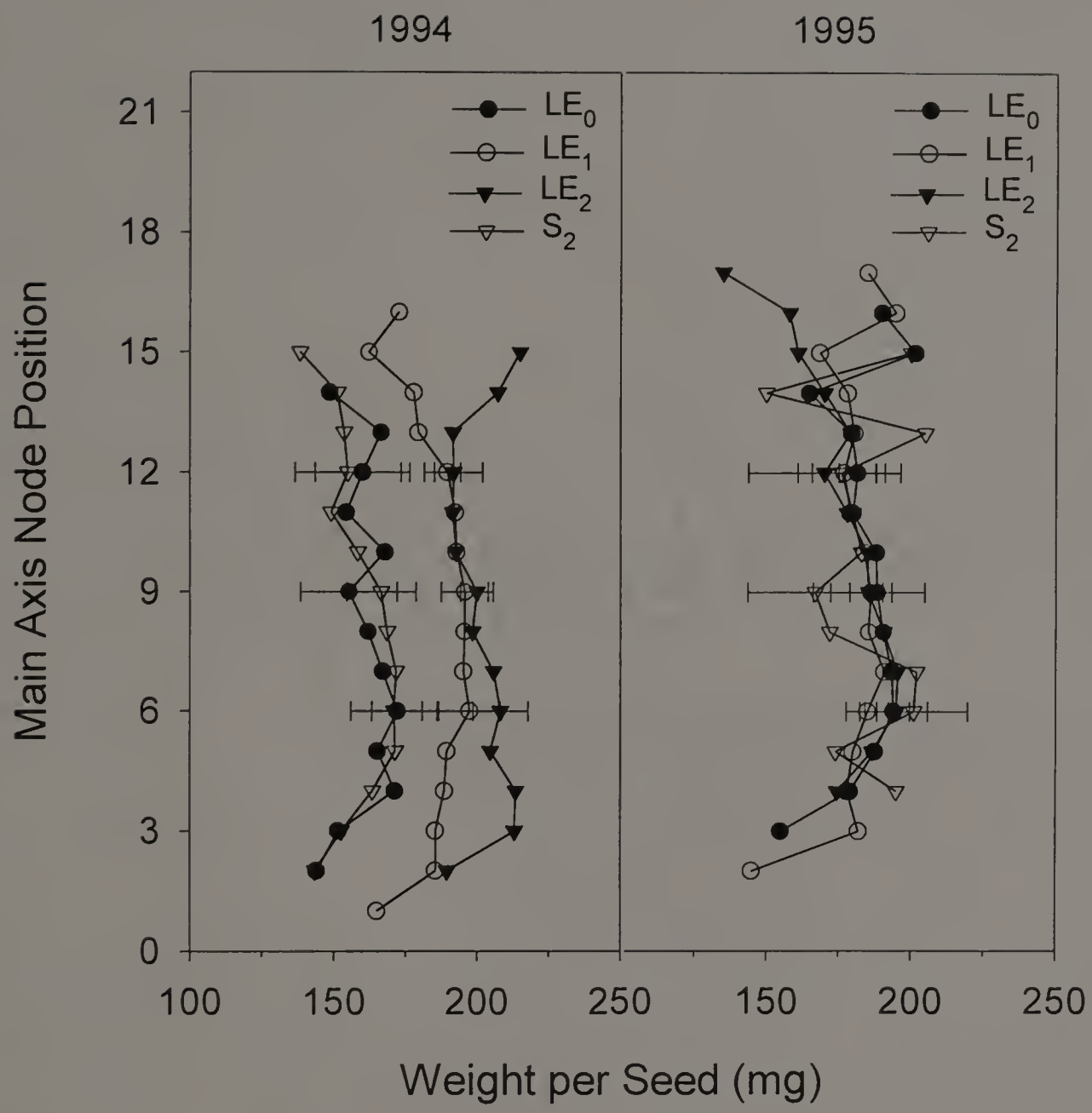

Figure 2.5 Seed weight per main axis node position in response to light treatments.

LE_0: Control; LE_1: Light enrichment beginning flowering; LE_2; Light enrichment at pod development; S_2: Shade at pod development.

caused a relatively sufficient source supply to the newly developing zygotes, embryos or seeds that otherwise might fail to develop under poor light conditions.

The size of seeds on a nodal basis for light treatments is shown Fig. 2.5. The size of the seed was relatively constant across the main axis of soybean plant within a treatment in 1994, except for extreme nod position in each light treatment. However, in 1995 there was some variability observed in seed sizes across the main axis of soybean plant. Seed size of soybean was a function of the rate and duration of dry weight accumulation in the seed fraction (Egli et al., 1978). Environmental factors which influence the rate of dry matter accumulation have a direct effect on yield.

Summary

The yield of Evan soybean can be enhanced by increasing light availability to the plant. Optimizing light condition during early reproductive growth stage (LE_1) was more effective in determining the final seed yield. The increase in yield under the light treatments were largely contributed by an increase in pod number per plant, although variations in seed size across the light treatments also partially contributed to an increased final yield in LE_2. These differences in seed sizes observed under light treatments can help explain yield variation between years at the same field location. Harvest index was enhanced by improvement in light condition in LE_1, showing an increase in partitioning of assimilates to reproductive sinks resulting in yield increases.

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CHAPTER 3

EFFECT OF LIGHT ENRICHMENT AND SHADING ON ENDOGENOUS ABSCISIC ACID CONCENTRATIONS IN SEED COMPONENTS, COTYLEDON CELL CHARACTERISTICS AND SEED SIZE IN SOYBEAN

Abstract

Seeds are the primary sinks for photosynthates during reproductive growth and an understanding of the factors that control the rate of seed growth in soybean (*Glycine max* (L.) Merrill) is necessary to understand the yield production process. Factors which affect seed development are ultimately reflected in the final yield through a variation in seed size (weight per seed) and number of seeds per unit area. The plant growth regulator, abscisic acid (ABA) has been implicated in the regulation of assimilate transfer within filling soybean seeds. Variations in seed size within a single genotype are observed between years, locations and when plants are grown with light enrichment or light reduction treatments during the period of seed development. We investigated the possibility that either a decreased cotyledon cell number or size along with changes in the endogenous abscisic acid levels in seed coat and cotyledon were responsible for differences in seed weight. Soybean plants were subjected to light reduction and enrichment treatments from the beginning of pod formation stage until the final harvest. Endogenous ABA levels in cotyledons and the seed coat of soybean were determined by Enzyme Linked Immunosorbent Assay (ELISA). Higher rates of seed growth and greater seed dry weight were observed with light enrichment. The levels of ABA in

cotyledons during seed development were significantly correlated with seed growth rates under the light treatments. Reduction in growth rate of seeds and cotyledon cell number, along with significant lowering of endogenous ABA levels in seed coat and cotyledon were observed with shade. Decreased light availability together with reduction in endogenous ABA levels in seed may decrease the assimilate availability during the critical cell-division period, thus regulating the seed growth rates for the remainder of seed filling period.

Introduction

Factors which affect seed development ultimately have an influence on the final yield by affecting the seed size (weight per seed) or number of seeds per unit area (Swank et al., 1987). These physiological processes controlling the rate of seed growth and final seed size are not completely understood. A comparison of the seed growth characteristics of several soybean genotypes has shown seed size to be more closely correlated with the growth rate than filling duration, both of which are under the genetic control (Egli et al., 1981). However, significant differences in seed size among soybean genotypes with no differences in growth rates are reported, which indicate that differences in seed size were associated mainly with a variation in duration of seed filling (Swank et al., 1987).

Seed size is affected by the number of cells per seed and cell size. Cell number had a greater influence on productivity than cell size in a number of crop species including wheat endosperm (Asana et al., 1969; Jenner, 1979),

barley endosperm (Cochrane and Duffus, 1983), corn endosperm (Jones et al., 1985) and sugarbeet tap root size (Doney et al., 1981). In soybean, genetic differences in seed growth rates were controlled by the developing seed and were related to number of cells in cotyledons (Egli et al., 1980). Cotyledon cell number was influenced by the physiological environment during cell division phase of seed development, and both cell number and assimilate availability were important in determining the growth rate of soybean seed (Egli et al., 1989).

Although genotypic differences in seed growth rates are related to number of cells in cotyledons of soybean, within a single genotype the final seed size may be influenced more by cell size than cell number (Hirshfield et al., 1992). Several studies have shown that unfavorable temperatures during endosperm cell division in wheat reduced the endosperm sink capacity by reducing cell size with little or no effect on cell number (Radley, 1978; Wardlaw, 1970). Similarly, genetically based increase in seed size in soybean were reported to be due to an increase in cotyledon cell size and longer seed filling periods but not to cell number per cotyledon (Swank et al., 1987). However, there is enough evidence that suggests both cotyledon cell number and cell size to be related to genotypic differences in seed size and consequently effect the final seed yield in soybean.

Evidence is available that plant hormones are involved in determining both sink size and its capacity. Abscisic acid (ABA), earlier thought of as an inhibitory substance, is now recognized as a naturally occurring plant hormone

of major importance in the regulation of many aspects of plant growth and development (Milborrow, 1974; Walton, 1980). Research shows ABA to be actively involved in regulation of transport and storage of assimilates during the grain development in a number of plants. Developing fruits contain in addition to cytokinins and auxins, a high concentration of ABA during the grain filling period (King, 1976). ABA levels rise sharply during seed development and then decline to low levels as seeds mature. This profile of ABA changes has been observed in wheat (King, 1976), pea (Eauwens and Schwabe, 1975), bean (Hsu, 1979), barley (Goldbach and Goldbach, 1977) and soybean (Quebedeaux et al., 1976).

Environmental conditions during seed development modify seed growth rate and final seed size, both of which though are genetically limited (Egli et al., 1985). Short day photoperiods were reported to increase the partitioning of assimilates to filling seeds of soybean which resulted in higher seed growth rates (Morandi et al. 1990). An earlier accumulation of ABA and sucrose in short day plants were reported and ABA was suggested to increase assimilate availability during the critical cell-division period, thereby regulating cotyledon cell number and seed growth rate.

Some reports concerning the effect of ABA on assimilate transport and distribution are also contrary to the above mentioned results. There is evidence that ABA lowers the assimilate-transport intensity as ions and assimilates have been found to move away from the site of ABA application (Mullins, 1970). In castor bean, an inhibition of sucrose uptake by the cotyledons occurred when

incubated in the presence of ABA (Vreugdenhill, 1983). ABA at lower concentration has been found to promote certain responses which are inhibited as the concentration is increased in the tissues (McWha and Jackson, 1976). It appears that ABA concentration changes vary with each species and its regulatory role in seeds differ in different species.

Immunological analysis is now a recommended method in quantification of ABA and related compounds. The enzyme-linked immunosorbent assay (ELISA) is based on competition between free ABA in the sample and an alkaline-phosphatase labeled ABA tracer for a fixed number of high-affinity antibody binding sites (Weiler, 1984). Through the use of a sensitive monoclonal antibody (McAb), quantitative measurements can be made on small tissue samples.

Traditional techniques for studying the effects of growth regulators on physiological processes are done by the application of the compound in study or by the inhibition of its synthesis or activity by chemical means. The main disadvantage of the exogenous application of a hormone is that it leads to its availability at the inappropriate place, time and or concentration and the techniques that are used to apply the hormones or their inhibitors can sometimes cause severe injury to the tissues (de Bruijn and Vreugdenhil, 1992). An alternative approach to exogenous application of plant growth substance to plant tissue were achieved by modifying the endogenous level of the growth substance in the target tissue. Photoperiodically induced changes

in seed growth rates in soybean were observed and related to the endogenous ABA concentration and sucrose in seed tissues (Morandi et al., 1990).

In this investigation endogenous ABA content of developing cotyledons and seed coats of soybean under light enrichment and shading conditions were examined. The main objectives were a) to determine if cotyledon cell number or cell weight and volume were responsible for the differences in seed size in soybean when subjected to light variation and to examine their relationship to endogenous ABA concentrations in cotyledons and seed coats and b) to determine if the rate of seed filling, effective filling duration or both were responsible for the changes in seed yield under different light treatments.

Materials and Methods

Cultural Practices

A 2-year field study was conducted in 1994 and 1995 in the Connecticut River Valley at University of Massachusetts Agricultural Experimental Station Farm in South Deerfield, Massachusetts. The soil type was a Hadley fine sandy loam (Typic Udifluvent, coarse-silty, mixed, nonacid, mesic). In 1994, the experimental site tested high for P, thus it only received an application of muriate of potash at a rate of 306.5 Kg K ha^{-1} prior to cultivation. No fertilizer was applied for 1995.

Evans, an indeterminate, Group 0 soybean, with approximately 92% seed germination rate was inoculated with commercial powdered-peat inoculant before planting. Seeds were machine planted on May 24, 1994 and May 23,

1995. The experimental design was a complete randomized block design with three replications and each plot size was eight rows wide by 12 m long. Tillage practices were mold-board plowing and disking prior to planting. Irrigation was not needed in either year.

Weed control in 1994 consisted of a post emergence mixture of 2.06 liter ha^{-1} poast [2-(1-(ethoxyimino)butyl)-5(2-(ethylthio) propyl)-3-hydroxy-2-cyclohexene] and 2.75 liter ha^{-1} crop oil. In 1995 weed control was done by pre-emergence applications of 1.7 kg ha^{-1} active ingredient (a.1) alachlor [2-chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide] and 0.85 kg ha^{-1} a.i. linuron [3-(3,4- dichlorophenyl)-1-methoxy-1 methylurea] plus hand weeding during the early vegetative growth stage of soybean.

Light Enrichment and Shading Treatments

Light enrichment (LE_2) entailed increasing solar radiation available to the center sample row of each plot at beginning of pod formation R stage. This was achieved by installing a 90 cm tall wire mesh fence 25 cm from each side of the sample row, sloping at approximately 45^0 angle away from it. These fences were inspected periodically, and the plants held back by the fence were rearranged to avoid undue crowding to allow near normal growth of these fenced border row plants. Plants from the neighboring rows were prevented from encroaching into the center sample row thus allowing greater light penetration into the canopy of the center sample row. This method was chosen to allow light penetration deeper into the canopy without disturbing the root

system of plants in border rows. Once put in place, these fences remained in position until soybean maturity. Shade (S_2) was provided by using black polypropylene fabric installed 0.5 m above the soybean canopy at the R-stage(LE_2), and remained in place until seed maturity. Shade cloths were attached to a vertically movable wire trellis attached to wooden posts. A control treatment (LE_0) received no light enrichment or shade.

Interception of Photosynthetically Active Radiation

Light measurements were taken above, within, and below the canopy, of the available photosynthetically active radiation (measured in microeinsteins per m^2 per s) for all the treatments at the beginning of pod development. Using a Li-Cor line quantum sensor (LI-188B), at 1100 to 1350 hours on eastern daylight time on days when clouds caused no interference. Inter-row light readings were taken with the light sensor placed across the inter-row space. All readings were integrated over 1m and 10 s.

Tissue sampling for ABA determination

Pods were sampled every week from 19 days after anthesis at respective nodes (beginning of the linear seed filling period -R) through 38 days after anthesis just before the seeds reached physiological maturity. Pods at node positions 4, 8 & 12 on stem of the soybean plant were sampled. Four middle positioned seeds from three seeded pods were pooled together from different plants for ABA analysis. Selected seeds were dissected into their

seed coat and cotyledon components. Fresh weights were recorded and the samples were immediately frozen on dry ice and stored at approximately -18^{0}C.

ABA Extraction and Quantification

The technique used for ABA extraction was similar to those described by Montero et al. (1994). The plant tissue was homogenized in liquid nitrogen for 5 minutes. It was then extracted overnight at 4°C in 80% aqueous methanol containing 1mg liter^{-1} of butylated hydroxytoluene to avoid oxidation. To remove any impurities, the methanolic extract was passed through a Sep Pak® C_{18} cartridge prewashed with 1 ml of 80% methanol. This methanolic phase was then evaporated in a vacuum oven at room temperature. The resulting aqueous portion was partitioned 3 times against ethyl acetate of pH 3.0. The ethyl acetate fractions were combined and evaporated to dryness under low pressure. The residue were dissolved in saline tris buffer (TBS) of pH 7.8 and then quantified by enzyme -linked immunosorbent assay (ELISA). A stock solution of 1 mM (±) ABA standard was prepared in absolute methanol and diluted in TBS pH 7.5. Concentrations ranging from 0-2 pmole μl^{-1} and 100 pmol μl^{-1} were used to form the standard curve. Tissue sample eluates were serially diluted in TBS pH 7.5.

Strips of flat bottomed reaction wells coated with ABA McAb were placed in a strip holder plate. To each reaction well, 100 μl of either diluted tissue sample or ABA standard were added. Then, 100 μl ABA-alkaline phosphate

conjugate in tris-buffered saline was added to each well. The holder plates were sealed and placed in dark at 4^0C for three hours for incubation. The solutions were decanted, and wells were washed with a tween-saline solution. The enzyme tracer activity was assayed by the addition of p-nitrophenyl phosphate substrate to each well. The holder plates were incubated at 37°C in an oven for 60 minutes and removed. One drop of 1N NaOH was added to each well to stop the reaction. Color absorbance was read at 405 nm.

To determine if there will be any interference in the assay from sample extracts, samples of plant extracts were added to increasing concentrations of ABA standards (Walker-Simmons, 1987). ABA standards alone and ABA standards plus extracts were assayed for ABA content and plotted as ABA added versus ABA found. Any interfering substance in the plant extract would change the slope of the curve (Pengelly, 1977). The degree of parallelism between the lines was evaluated.

Seed Growth Rate and Effective Filling period

Pods and seed samples were collected from the same plants used for ABA quantification every 5-6 days during the linear phase of growth. Three seeded pods from node positions 4, 8 and 12 were collected to minimize any bias from the position of the seed in pod. After the samples were collected, seed fresh mass was determined and seed volume was determined by water displacement method. Seeds were dried at approximately 70^0 C for 24 hour and the moisture content calculated. Linear regression was used to estimate

seed growth rates for each treatment, after eliminating the nonlinear points from initial and final stages of seed development. At maturity, plants were sampled to determine the final seed size. The effective filling period (EFP) was estimated by dividing final seed size by rate of dry matter accumulation in seeds during the linear filling period. (Daynard et al., 1971).

Cotyledon Cell Number and Size

The number of cotyledon cells was estimated using the method described by Swank et al. (1987). Seeds were dried for 24 hours (70^0 C) and then allowed to imbibe water for 8-10 hours, after which the seed coat and embryo axis were removed. One cotyledon per seed was placed in a fixative formalin acetic acid solution (Berlyn and Miksche, 1976). Then, 24 hours in formalin acetic acid, each cotyledon was cut finely and digested in 40 ml of chromic acid solution (80g chromic acid liter $water^{-1}$) (Reinert and Yeoman, 1982). The chromic acid was removed after 5 days, and cotyledons were macerated before diluting with water. An aliquot of the cell suspension was placed on a hemacytometer, and the cells were counted under X 100 magnification. For the material not completely digested, the suspension was filtered, and residues were dried and weighed. The number of cells counted was adjusted by the proportion of the total cotyledon mass not digested to give total number of cells in the cotyledon. Cell growth rate was calculated by dividing the seed growth rate by cell number. Cell volume was obtained by dividing maximum fresh seed volume by number of cotyledon cells. The

estimated number of cells per cotyledon pair were calculated using the known volumes.

Seed Yield and Yield components

Final yield was determined by harvesting plants in the central 4 rows with a length of 3m for LE_0 and S_2 , while for LE_2 3m of the central row between the fences was harvested. For the yield components, 15 plants in each plot under different light treatments were harvested at plant maturity and data were recorded for the whole plant as well as for each node position on the main axis. The data recorded were the pod number, seed number, stem dry weight, pod dry weight and seed dry weight. From these the seed yield components were calculated.

Results and Discussion

Light interception

Light intensity at the top of canopy at beginning of pod formation stage was 13.06 and 7.34 $uEs^{-1}m^{-2}$ for the unshaded and shaded treatments respectively (results averaged over 1994 and 1995 - Fig. 2.1). At the base of canopy it was 0.19, 3.37 and 0.03 $uEs^{-1}m^{-2}$ for LE_0, LE_2 and S_2 respectively (Fig 2.2). Shading resulted in a 52% light reduction compared to the control. Thus 98.6% and 74.3% of incoming solar radiation was intercepted by the soybean crop canopy for LE_0 and LE_2 respectively. The leaves at the base of canopy in LE_2 were receiving more than 25% of the available light.

Rate and duration of seed filling period

There were significant differences observed among the treatments in seed growth rates in both the years (Table 3.1). The final seed weight (size) in soybean is determined by the rate of seed growth and duration of seed fill, both of which partially are under the genetic control (Egli et al., 1984) although the environment can modify these seed growth components (Egli et al., 1985, 1989). The light treatments had a large effect on the pattern of seed growth during the filling period and final seed weight at maturity (Fig 3.1 & 3.2). The rates of seed growth were higher in LE_2 than the control, while S_2 resulted in significant lowering of seed growth rates compared to controls in both years (Table 3.1). These differences in seed growth rate were significantly correlated with the final size of the seed at maturity ($r = 0.99^{***}$, 0.83^{**} in 1994 and 1995 respectively). Seed growth rate in soybean, rather than filling duration has been positively correlated with the seed size (Egli et al., 1981; Guldan and Brun, 1985)

A negative correlation was observed between the seed weight and duration of seed filling as the time of seed fill was significantly increased under the shade treatments. There was a significant increase of about 73% in the filling duration of seeds due to the shade environment in 1995, while differences were non-significant in 1994 (Table 3.1). In contrast to the above results, Swank et al. (1987) reported that a close relationship between final seed size and growth rates of seeds does not hold true for all soybean genotypes as they found a variation in duration of seed fill to also make a

Table 3.1 Seed growth characteristics of soybean in response to light variation treatments across years.

Treatment	Seed Growth Rate (mg seed^{-1} day^{-1})		Effective Filling Period (days)	
	1994	1995	1994	1995
LE_0	10.86 b	13.82 b	15.81 a	13.45 a
LE_2	13.04 a	17.25 a	15.52 a	11.61 a
S_2	8.34 c	8.35 c	18.98 a	23.28 b

- Data points are the means of seeds from 4 different plants and averaged over 4 replications.
- LE_0, LE_2 and S_2 represent control, light enrichment and shade treatments respectively.
- Means within columns followed by the same letter are not significantly different at the 0.5% level of probability using Duncan's Multiple Range Test.

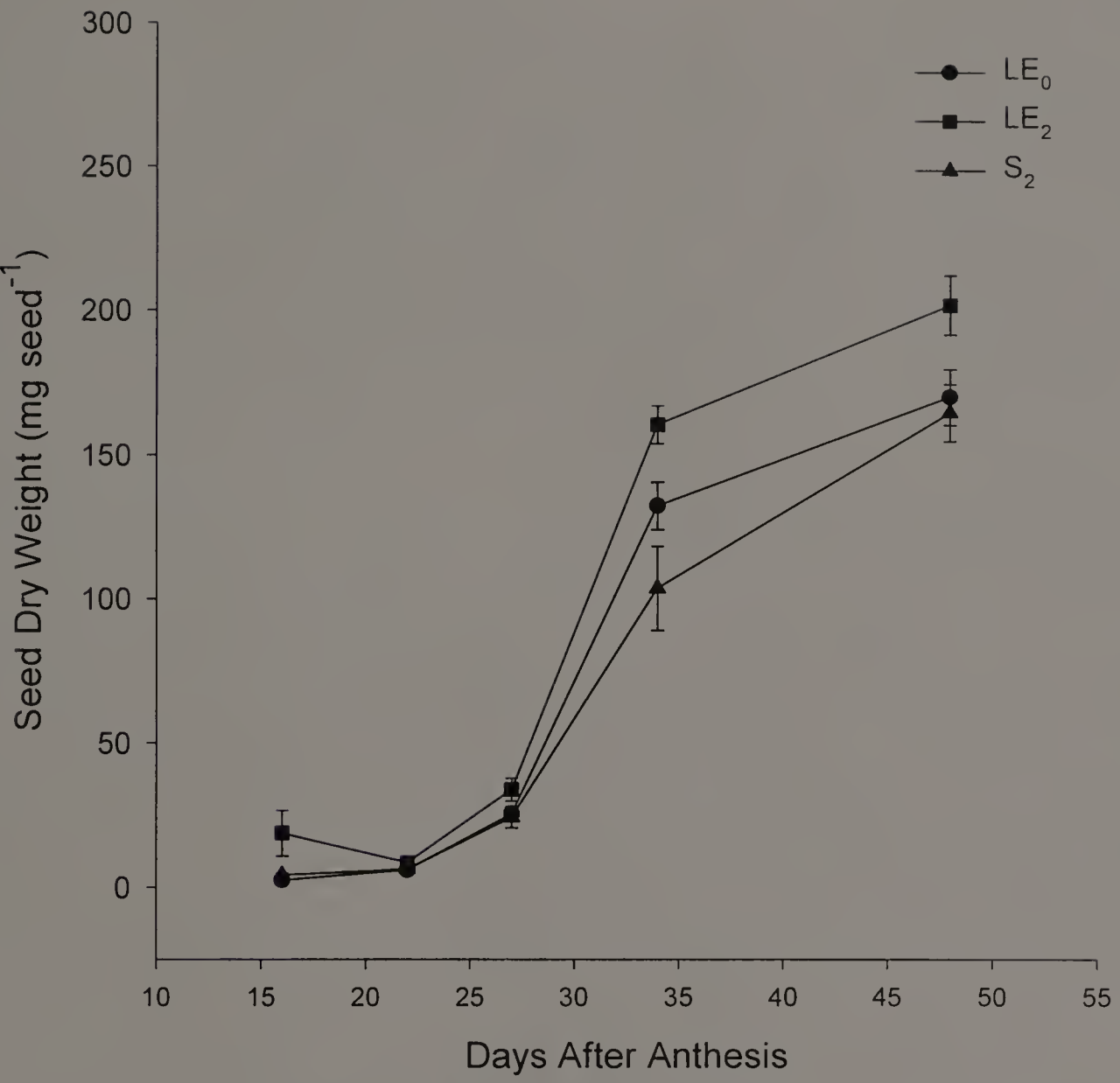

Figure 3.1 Effects of light enrichment and shade on seed dry weights during the seed development in soybean in 1994.

LE_0, LE_2 and S_2 represent control, light enrichment and shade treatments respectively.

- Data points are average of 4 replications.
- Vertical lines represent standard error of mean.

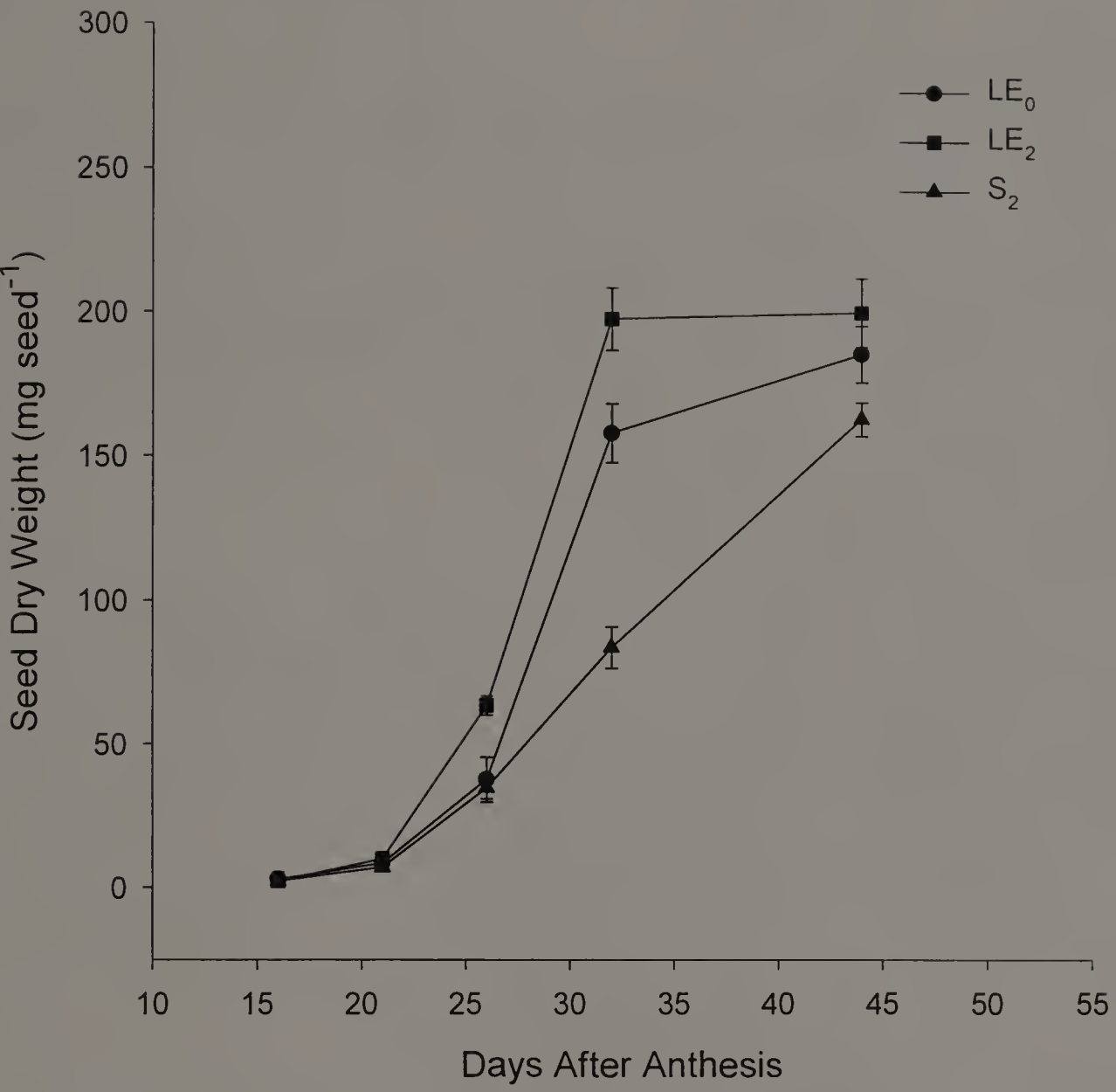

Figure 3.2 Effects of light variation on seed dry weight during the seed development in soybean in 1995.

LE_0, LE_2 and S_2 represent control, light enrichment and shade treatments respectively.

- Data points are average of 4 replications.
- Vertical lines represent standard error of mean.

significant contribution to the differences in seed size. These results demonstrate that a variation in light interception by soybean crop canopy can influence growth rates as well as filling duration of seeds which ultimately affects the seed size.

Cotyledon Cell Characteristics

Cotyledon cell number was sensitive to light condition during flowering and seed development and differed under the light treatments (Fig 3.3 & 3.4). The final cell number in the cotyledons of soybean plants under shade treatments were reduced by 30% and 19% in 1994 and 1995 respectively. Shading soybean plants during seed development could have resulted in a reduction of assimilate availability, thus affecting the cell division in the cotyledons. Egli et al. (1989) found that assimilate reduction treatments, when imposed during the initial stages of seed development reduced cell division and cell number in the cotyledons and resulted in reduced seed growth rates. They manipulated the source-sink ratio by shading or defoliation during initial stages of seed development and consistently found a reduction in cotyledon cell number in all genotypes tested. On increasing the source-sink ratio by fruit removal there was an increase in cotyledon cell number. It has been reported that low irradiance (Wardlaw, 1970; Brocklehurst, 1977) and moisture stress (Brocklehurst et al., 1978) have reduced the number of cells in endosperm of wheat kernels.

The growth rate of cells in the cotyledons ranged from 1.02 to 1.86 ng day^{-1} across years and treatments (Table 3.2). The rate of cell growth differed in all the three treatments and was significantly correlated with seed dry weight at maturity (0.93***) and rate of seed growth (0.99***) in 1995 only and not in 1994. Swank et al. (1987) reported significant differences in cotyledon cell number in one soybean genotype across years and suggested that environment may have influenced the number of cells in developing cotyledons of seeds. Variations in the growth rate per cell probably reflect variations in the supply of assimilates to the seed since this affects the growth rates of seeds in soybean (Egli et al., 1985).

Differences in cell number among treatments were significantly correlated with seed dry weight at maturity in both years. Previous authors have also reported a close association between cotyledon cell number and growth rates in soybean (Guldan and Brun, 1985; Egli et al., 1981). Cell number in soybean is under genetic control and can be influenced by physiological environment of seed during the cell division phase of seed development. Growth rate of seeds in soybean is a function of the number of cells in the cotyledons and the supply of assimilates to the developing cotyledons (Egli et al. 1989).

There were no significant differences in cell weight or cell volume in cotyledons of the seeds in the three treatments (Table 3.2). In contrast to these results, variations in cotyledon cell size are known to be associated with differences in seed size in differing soybean genotypes. As the seed size increased the number of cells per unit mass of seed decreased, showing cell

size to mainly contribute to variation in seed size (Swank et al. 1987). The results of our experiments showed that variations in seed size within one genotype due to varying light treatments were a result of differences in the cotyledon cell number and not cell size.

ABA levels in seed coats and cotyledons

To evaluate ABA distribution within developing seed, seeds were harvested approximately at weekly intervals post-anthesis, dissected and carefully separated into seed coats and cotyledons. ABA could be detected in all cotyledons and seed coats extracts sampled throughout the course of seed development. Data of ABA concentrations of soybean cotyledon and seed coat from 16 days post-anthesis to maturation are presented (Figure 3.5). The ABA concentration in these seed components decreased from a measured high 16 days after anthesis as seeds accumulated dry mass. The quantification of ABA concentration prior to this time was not possible as it was difficult to separate the seed into seed coat and cotyledon components because of small seed size.

The ABA levels and concentration changes for developing soybean cotyledons and seed coats differ from those reported for other crop species. In cotton fruits, rapid increases in ABA levels were correlated with young fruit abscission and then at fruit maturation during the time of wall senescence (Davis and Addicott, 1972). Similarly, in grape berries high level of ABA accumulated at fruit ripening (Coombe and Hale, 1973). In wheat maximum ABA accumulation in developing seeds occurred during the most active growth

Table 3.2 Cotyledon cell characteristics of soybean in response to light treatments across years.

Treatment	Cotyledon Cell Volume (μl X 10^{-6})	Growth Rate Cell^{-1} (ng day^{-1})		Cotyledon Cell Weight (ng)	
	1995	1994	1995	1994	1995
LE_0	14.21 a	1.424 a	1.603 b	17.6 a	21.5 a
LE_2	13.46 a	1.429 a	1.863 a	17.14 a	21.6 a
S_2	16.91 a	1.555 a	1.020 c	19.47 a	23.2 a

- Data points are means of 3 replications.
- LE_0, LE_2 and S_2 represent control, light enrichment and shade treatments, respectively.
- Means within columns followed by the same letter are not significantly different at the 5% level of probability using Duncan's Multiple Range Test.

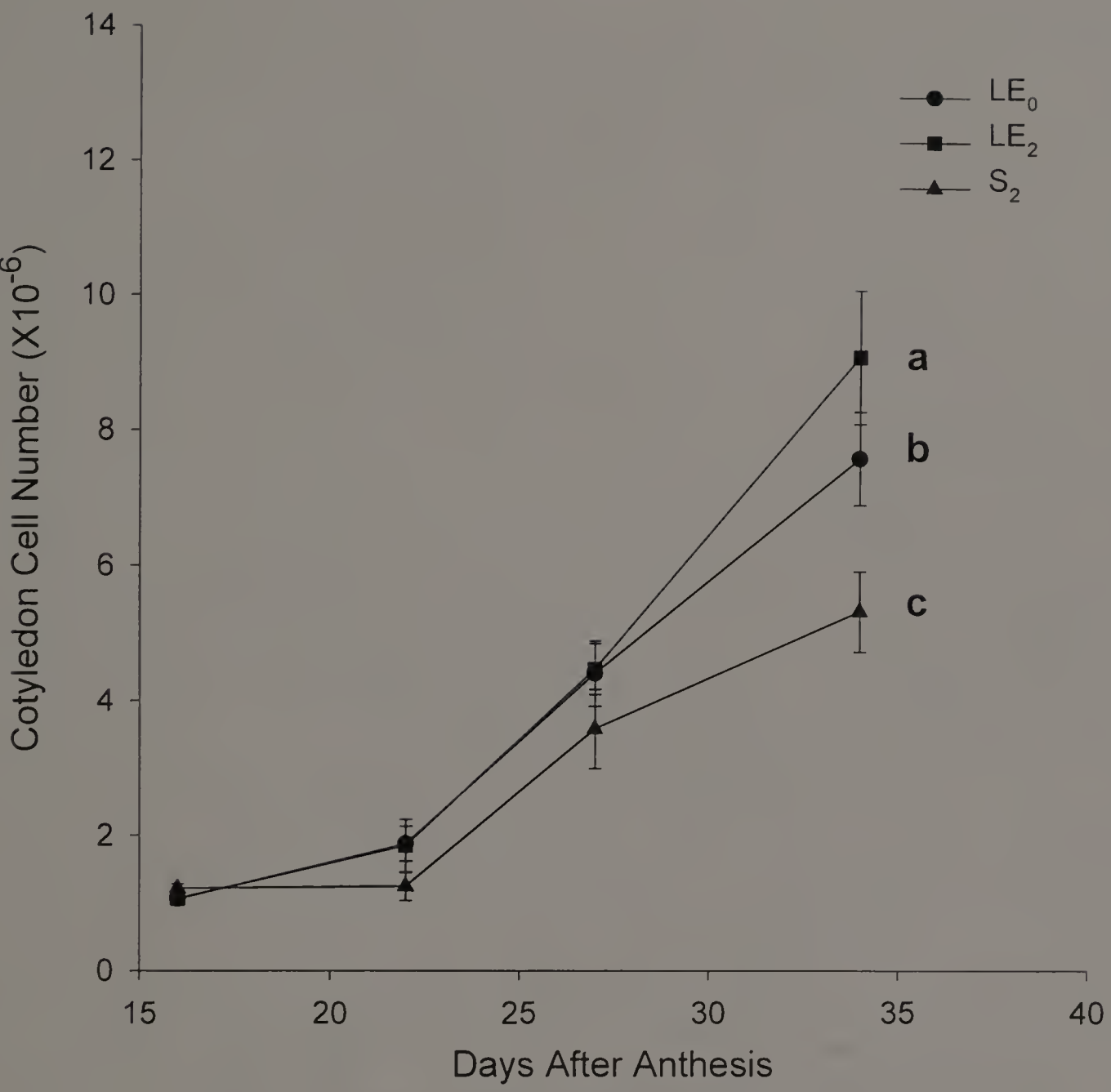

Figure 3.3 Effects of light variation on cotyledon cell number in soybean during the stages of seed development in 1994.

LE_0, LE_2 and S_2 represent control, light enrichment and shade treatments respectively.

- Data points are mean values of 3 replications.
- Vertical lines represent standard error of mean.
- Means followed by the same letter are not significantly different at the 5% level of probability using Duncan's Multiple Range Test

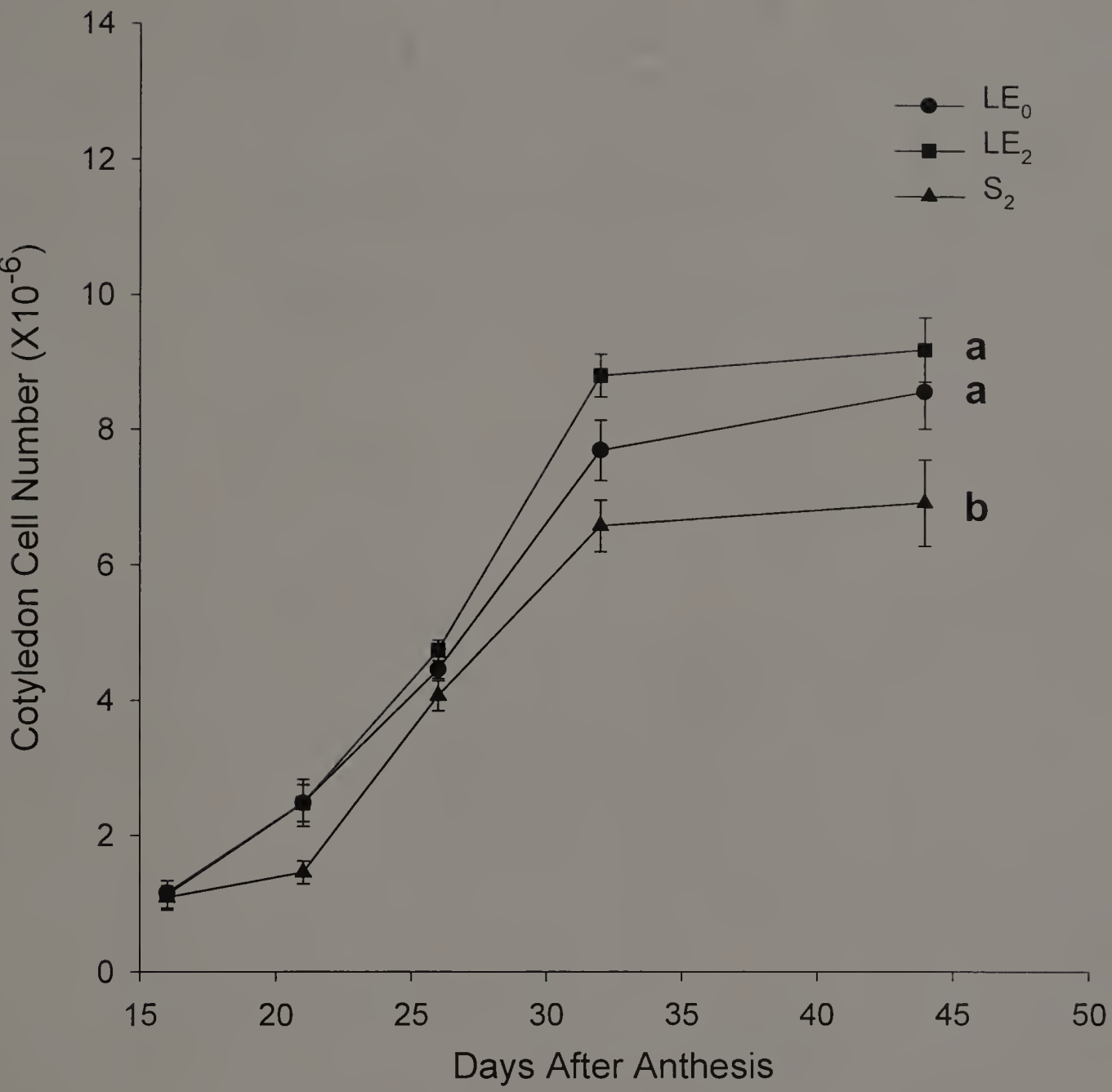

Figure 3.4 Effects of light variation on cell number per cotyledon pair in soybean during the stages of seed development in 1995.

LE_0, LE_2 and S_2 represent control, light enrichment and shade treatments respectively.

- Data points are mean values of 3 replications.
- Vertical lines represent standard error of mean.
- Means followed by the same letter are not significantly different at the 5% level of probability using Duncan's Multiple Range Test

period, which is similar to soybeans but the level was much less (McWha, 1975; Quebedeaux et al, 1974). The maximum ABA concentration, however, vary with each species and its regulatory role in seed development differs for each crop species.

In this investigation soybean seeds showed a higher concentration of ABA in cotyledon as compared to seed coat through out seed development. In soybean, seed coat and embryo function primarily as transport and storage tissues respectively (Morandi et al., 1990). These unique functions suggest that ABA and sucrose may be partitioned differentially between these seed tissues (Schussler et al., 1984). Similarly, a higher level of ABA concentration in cotyledons as compared to seed coats and root -shoot axis of soybean has been reported by Quebedeaux et al. (1976). However, there are reports where a higher concentrations of ABA in seed coats of soybean were observed as compared to developing embryos (Ackerson, 1984). Thus ABA is not equally distributed within the seed and this could be due to its different rate of biosynthesis, metabolism and or accumulation.

Effect of light treatments on endogenous ABA levels in seed coats and cotyledons

The ABA concentration in the seed components under the light treatments at 16 days after anthesis are presented (Table 3.3). There were no significant differences in the ABA concentration in seed coat and cotyledon of plants grown under LE_0 and LE_2, while light reduction resulted in significant

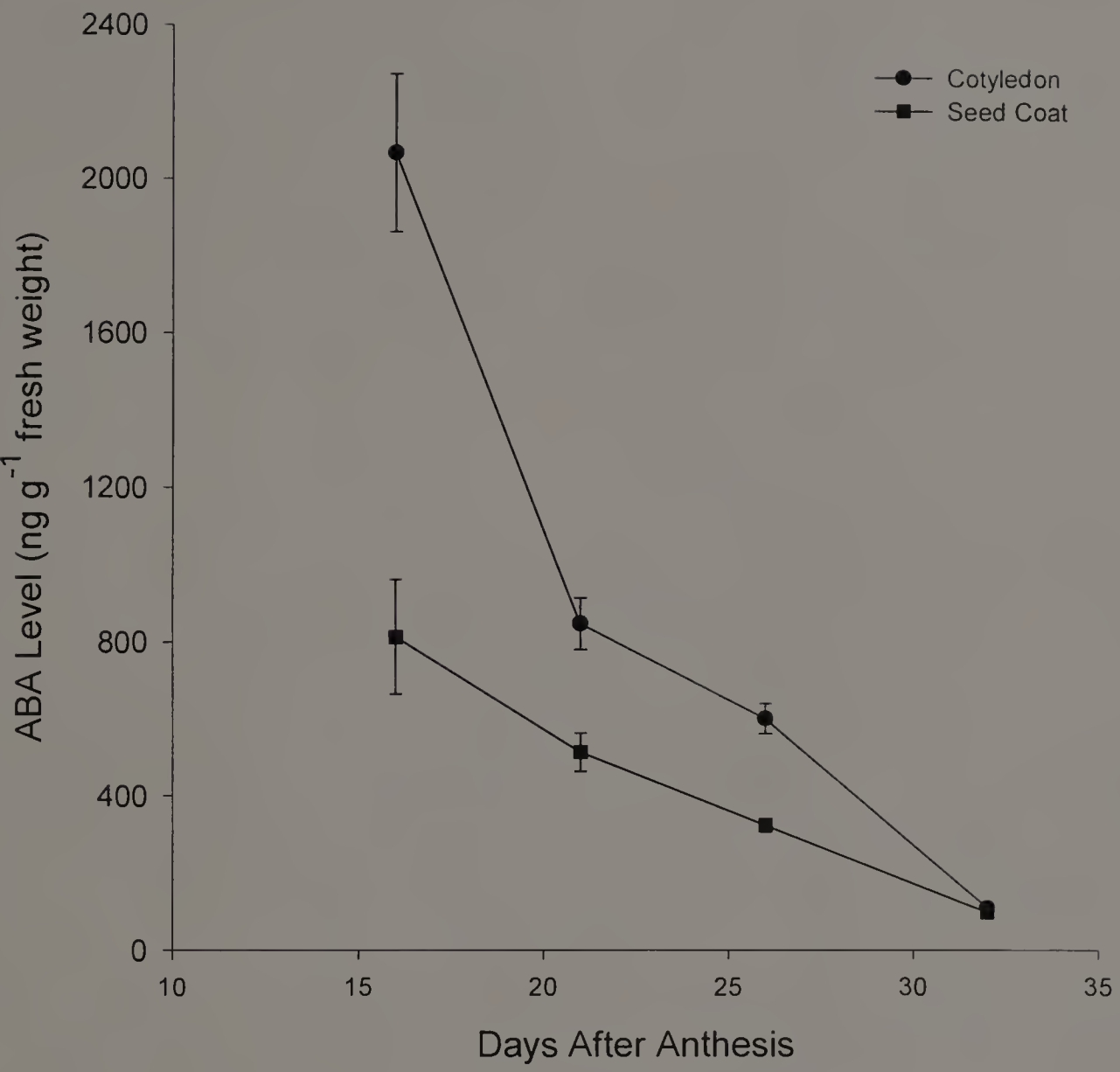

Figure 3.5. Abscisic acid concentrations in soybean seeds coats and cotyledon pair in relation to time after flowering in 1995.

- Data points are means of 3 replications.
- Vertical lines represent standard error of means.

lowering of ABA levels in cotyledon pair and seed coats (16% and 7% respectively about 16 days after anthesis as compared to the control) and throughout the seed development. ABA concentrations were high around 16 days after anthesis and then decreased with time in cotyledons and seed coats under the light treatments (Fig. 3.6 & Fig 3.7). ABA is reported to be synthesized in soybean leaves and exported in substantial quantities via the phloem (Setter et al., 1981; Hein et al., 1984). In lupin, ABA has been found to be exported from all the expanded leaves of the plant except the youngest and the apical bud (Wolf et al., 1990). The kernels of barley plants grown in the field showed higher endogenous ABA concentrations especially during the periods of high sunlight intensities (Tietz et al., 1981). This supports that most of the ABA in plants originates in leaf tissue and that any interference with the photosynthesizing leaves will lead to a reduction in ABA level in developing soybean seeds.

Seed growth rate of soybean was significantly correlated to the ABA levels in the cotyledons (0.89**) and seed coats (0.76*). Additional reports have correlated rapid increases in the rate of fresh and dry weight accumulation of the soybean seed with a high level of endogenous ABA levels in seeds (Quebedeaux et al., 1976). Higher concentration of ABA in the cotyledons of soybean genotypes were found to coincide with rapid uptake of sucrose, while lower rates of sucrose later during the seed filling period were found to be paired with lower concentration of ABA in the cotyledons (Schussler et al.,

1984). This supports that ABA concentration in seed tissue is involved in the rate of dry matter accumulation.

Previous research has reported that subjecting soybean plants to short day photoperiods resulted in earlier accumulation of ABA and sucrose, suggesting that ABA may have increased assimilate availability during the critical cell division period, thus regulating cotyledon cell number and seed growth rates for the remainder of seed filling period (Morandi et al., 1990). Similarly, there are findings which suggest that ABA could regulate the extent of cell division occurring during the early embryo development, thus influencing potential cell storage capacity (Ackerson, 1984) as ABA was found to accumulate at the time of cell division and when the DNA and RNA synthesis is extensive in developing Wye soybean cotyledons. Rapid increases in ABA accumulation in pea embryos were related to an increase in fresh weight accumulation of the cotyledons, mainly by cell expansion (Browning, 1980), showing a significant role of endogenous ABA concentrations in the cell-division phase of seed development in soybean.

Evans soybean is an indeterminate plant which continue vegetative growth when some parts of the plant become reproductive. Complex mechanisms are involved in the process of assimilate partitioning during the grain filling period due to different sources, like leaves, green stem parts and pod walls, which can contribute photosynthates to the developing seeds. The results show that the differences in the seed size among treatments were associated mainly with a variation in seed growth rates and cotyledon cell

Table 3.3 Distribution of ABA in developing soybean seed components sampled at 16 days post anthesis in 1995.

Treatments	ABA Concentration ($ng\ g^{-1}$ fresh weight)	
	Cotyledon Pair	Seed Coat
LE_0	2188 a	836 a
LE_2	2186 a	829 a
S_2	1832 b	777 b

- Data points are the means of seeds averaged over 3 replications.
- LE_0, LE_2 and S_2 represent control, light enrichment and shade treatments, respectively.
- Means within columns followed by the same letter are not significantly different at the 5% level of probability using Duncan's Multiple Range Test.

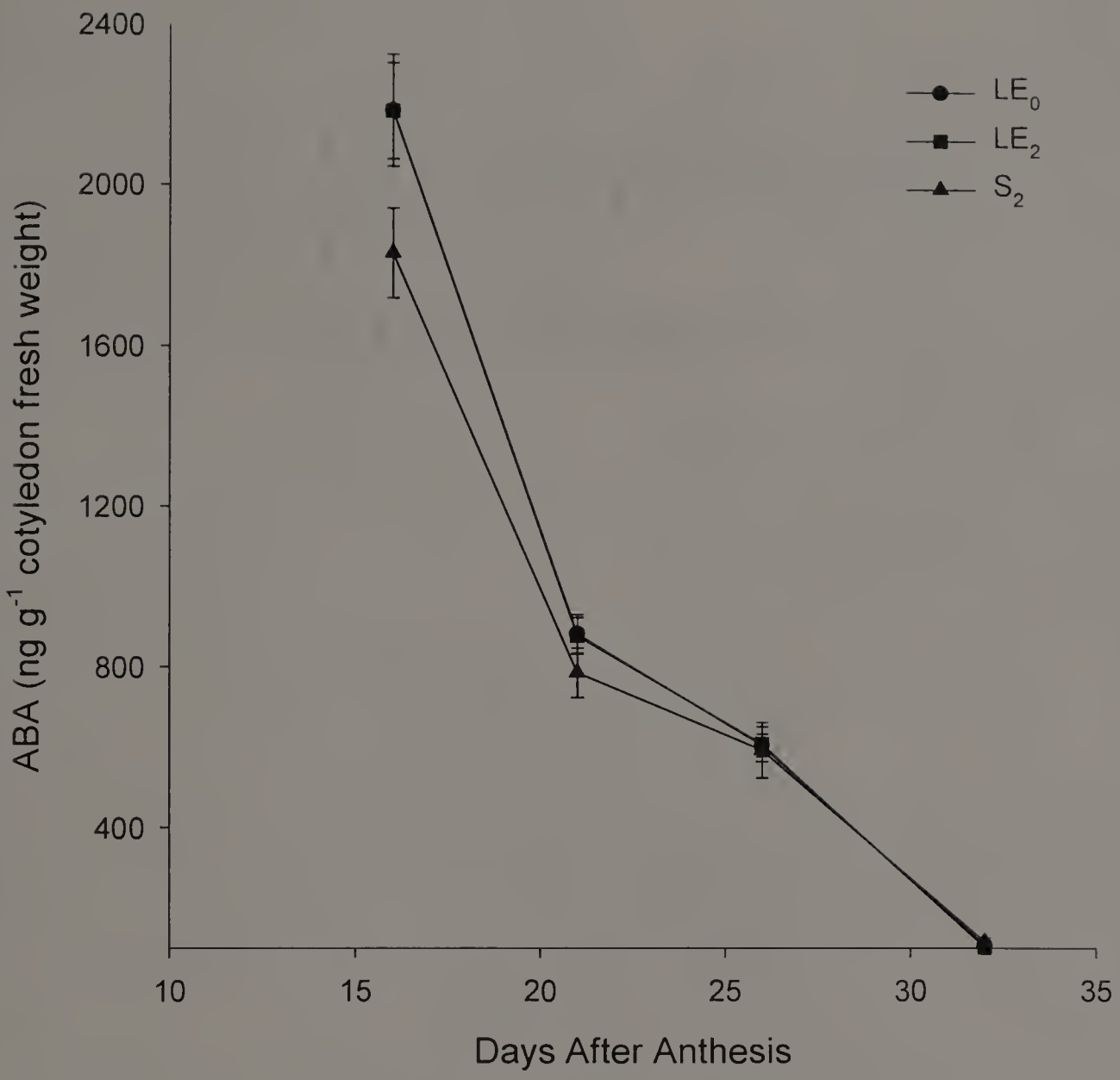

Figure 3.6 Abscisic acid concentration in seed cotyledons in relation to time after flowering in 1995.

LE_0, LE_2 and S_2 represent control, light enrichment and shade treatments, respectively.

- Data points are the means of seeds averaged over 3 replications.
- Vertical lines represent standard error of means.

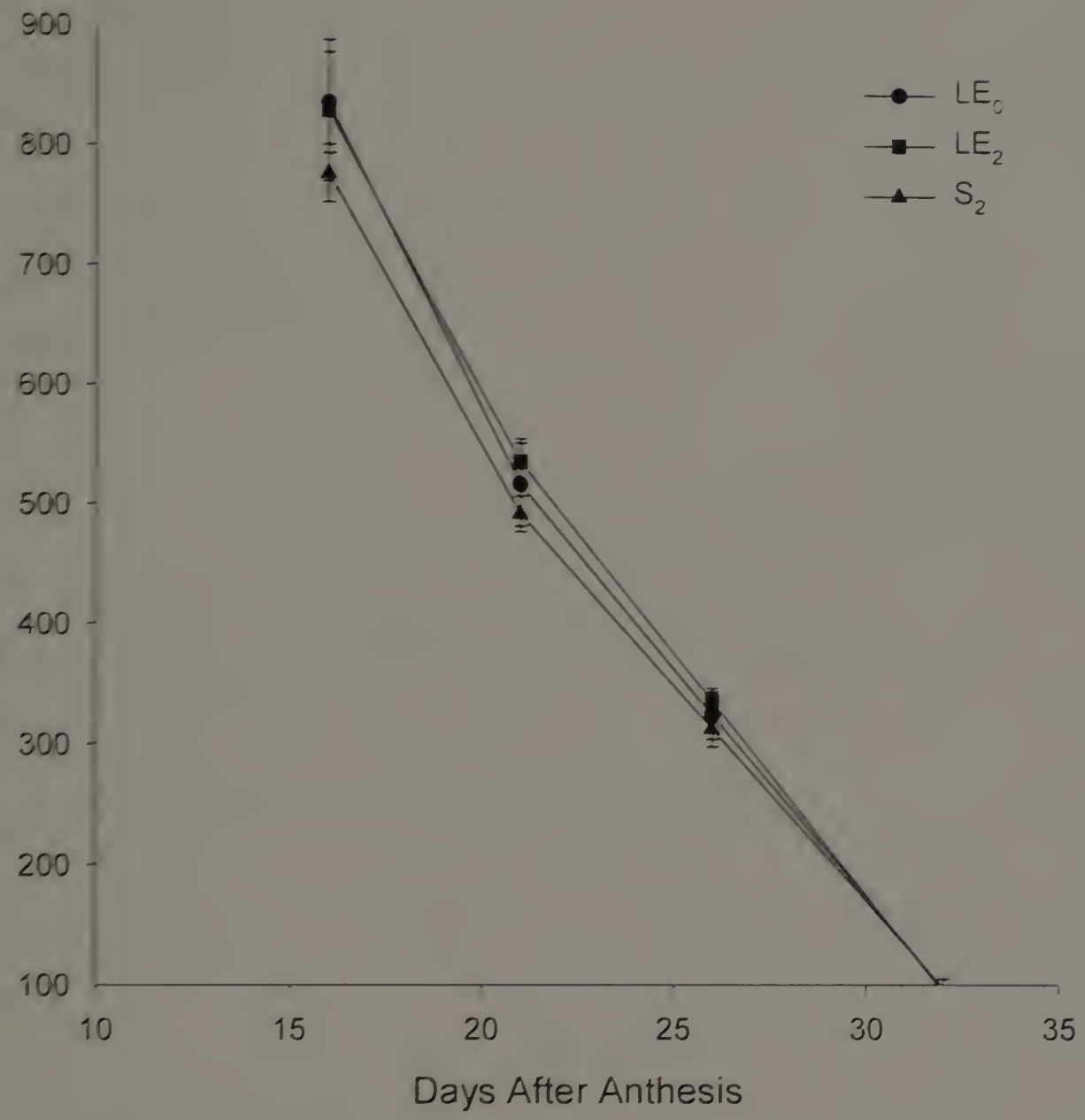

Figure 3.7 Abscisic acid concentration in seed coats in relation to time after flowering in 1995.

LE_0, LE_2 and S_2 represent control, light enrichment and shade treatments, respectively.

- Data points are the means of seeds averaged over 3 replications.
- Vertical lines represent standard error of mean.

number, which were higher for the plants subjected to light enriched environment. Mechanisms involved in these processes are unclear as additional information is needed on the influence of environmental factors on endogenous ABA levels during this period of seed growth and pod development and their relationship to seed growth rates and duration of growth.

Summary

The rate of seed growth is a function of the cell number in cotyledons and the assimilate supply to the developing cotyledons. Variations in seed sizes are observed when soybean plants are grown under varying light treatments. Higher rates of seed growth and greater seed dry weight were observed in plants grown under light enrichment. Results show that the actual number of cotyledon cells formed can be affected by the light interception by the soybean canopy, thus the potential sink capacity of the seed can be modified by the light availability during the early phases of seed development. ABA content of developing seeds was high during the initial stages of seed growth and development and significantly correlated with the seed growth rates in soybean under the light treatments. Plants subjected to shade early in the seed development had a lower rate of seed growth along with low concentration of ABA in seed components as compared to light enriched and control treatments. This decrease in light availability together with lowered ABA levels may have led to a decrease in assimilate availability during the critical cell-division period, thus regulating the rates of seed growth for the remainder

of seed filling period. Additional studies which can evaluate interaction between ABA, light environment and assimilate supply need to be conducted to improve our understanding of the regulation of soybean seed growth and development.

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CHAPTER 4

VARIATION IN SEED SIZE AND COTYLEDON CELL NUMBER IN RELATION TO SEED POSITION WITHIN PODS IN SOYBEAN

Abstract

Seed growth and development are important parts of yield, and information on factors which influence the rate and duration of seed growth in soybean (*Glycine max* (L.) Merrill) is needed to understand the yield production process. This study was undertaken to determine the variation in seed size within pods at different node positions on the main axis of soybean plant. Field studies were conducted to characterize the rate of seed growth and filling duration of seeds in relation to their position on plant. Cotyledon cell number, cell weight and cell volume were determined in seeds, as these parameters are known to be related to genotypic differences in seed size and final seed yield of soybean. The dry weight of the basal seed in three-seeded pods was significantly lower than the middle and terminal seed and it lagged behind in both fresh and dry mass accumulation through out the seed development. The significantly higher rates of seed growth of middle and terminal seed in pods as compared to the basal seed may have resulted in differences in final seed dry weight. The rate of seed growth is, therefore, partially determined by the genetic constitution of the seed. Non-significant differences in the filling duration of seeds within the pod were observed. Determination of cell number in cotyledons of basal seed showed lower number of cells per unit mass as

compared to the middle and terminal seed. The dry seed weight of soybean was significantly correlated with the number of cells in the cotyledons (0.78*), indicating cell number to be associated with differences in seed size within pod. Thus, within a single genotype, seed size variation within a pod may be influenced more by the rate of seed growth and cell number of the cotyledons than filling duration and cell size.

Introduction

The final seed yield in soybean (*Glycine max* (L.) Merrill) is a function of both the rate and duration of accumulation of dry weight by individual seeds. An understanding of the factors which control sink activity during the reproductive growth is necessary to understand the yield production process. The physiological factors which control the ability of seeds to accumulate dry matter are not completely understood. Seed dry mass is found to increase slowly after fertilization followed by a period of rapid growth at a constant rate of dry matter accumulation. This is followed by the final lag phase during which the rate of seed growth declines to zero as the seed reaches physiological maturity (Egli, 1975, Fraser et al., 1982).

In soybean, both the rate of seed growth and duration of seed fill are under genetic control of the plant (Egli et al., 1984), though these processes are also influenced by the environment (Egli and Wardlaw, 1980; Meckel et al., 1984) and by the supply of assimilates to the seed during development (Egli et al., 1985). Significant differences in seed size among soybean genotypes were

associated with a variation in duration of seed filling rather than with differences in the seed growth rates (Hanway and Weber, 1971; Egli and Leggett, 1973; Swank et al., 1987). There is evidence that different seed sizes in three soybean plant introductions were mainly due to differences in seed growth rates, with little variation in the filling duration (Guldan and Brun, 1985).

During endosperm cell division a potential sink capacity is established, and is determined by the number or size (or both) of the endosperm cells or starch granules formed or a combination of these factors (Capitanio et al., 1983; Reddy and Daynard, 1983). Across a wide range of plant species, seed size has been positively associated with rate of dry matter accumulation and cell number in cotyledons or endosperm. A positive relationship between cotyledon cell number and final seed weight in peas were observed (Davies, 1975). In sugarbeet tap root, a greater number of cells resulted in greater assimilate accumulation as an increased cell surface area led to an enhanced capacity for carbohydrate uptake (Doney et al., 1981). The growth rate and size of the wheat kernels were associated with number of cells in the endosperm (Brocklehurst, 1977).

Guldan and Brun (1984) demonstrated a positive correlation between cotyledon cell number and the ability of soybean seeds to accumulate dry matter. In contrast to the above results, Hirshfield et al., (1992) reported that within a single genotype, seed size may be influenced more by the size of the cotyledon cells than the cell number. According to Swank et al. (1987) larger

seed size in soybean genotypes was associated with greater cotyledon cell number and cell size, both contributed to the differences in seed size.

An indeterminate soybean plant usually blooms first at the unifoliate or second main axis node. Flowering then progresses upwards, and many new leaves and branches in leaf axils (especially at low plant densities) are developed concurrently with the progression of flowering. Well formed pods occur at the lower nodes before the last flower appears at the uppermost nodes. At final maturity, however, seeds at various node positions tend to reach the same size (Herbert and Litchfield, 1982). Seed number within a pod varies from 1 to 4 seeds, usually averaging between 2 and 3 seeds. In a study of 23 soybean genotypes, the basal seed which is the closest to the vascular connection was reported to be significantly smaller at maturity than the middle and terminal seed in both two-seeded and three-seeded pods (Herbert and Zhi-yi, 1984). The effect of differences in seed number and position of seeds in pods on the size of seeds in soybean is not well documented.

The objective of this study was to determine if differences in seed number per pod influenced seed size and if variation in seed size was related to seed growth rates, filling duration of seeds, cell number and / or cell size in developing soybean seeds.

Materials and Methods

Field Culture and Plant Material

Experiments were conducted in 1982 and 1983 (Herbert et al., 1984) and in 1995 in the Connecticut River Valley at the University of Massachusetts Agricultural Experimental Station Farm in South Deerfield, Massachusetts. The soil is a Hadley fine sandy loam (Typic Udifluvent, coarse-silty, mixed, nonacid, mesic). In 1982 and 1983, a basal application of 30 kg P ha^{-1} and 23 Kg K ha^{-1} was applied prior to planting. In 1995, the experimental site tested high for P and K and no fertilizer was applied.

Twenty three soybean genotypes were planted (Herbert et al., 1984) on May 18, 1982 and May 20, 1983 in 25cm rows at a density of 90 seeds m^{-2} in a randomized block design with 3 replications. Normal cultural management practices were followed; the soybeans were inoculated with a granular soil applied, peat based inoculum and weed control was achieved through the use of alachlor and linuron (Lasso and Lorox).

In 1995, Evans, an indeterminate, Group 0 soybean, with approximately 92% seed germination rate was machine planted on May 23 in 1995 at a density of 83 seeds m^{-2} in a randomized complete block design. Plots consisted of eight 25 cm rows 10 m long. Soybean seeds were inoculated with a commercial powered-peat inoculant in field experiments except in 1995 when soybean had been grown on the experimental area the previous year, so nodulation was effective in all years. Tillage practices were mold-board plowing and disking prior to planting.

Weeds were controlled by a pre-emergence application of 1.7 kg ha^{-1} active ingredient (a.i.) alachlor (2-chloro-2', 6'-diethyl -N- (methoxymethyl) acetanilide) and 0.85 kg ha^{-1} a.i. linuron (3-(3,4-dichlorophenyl)-1-methoxy-1 methylurea). Irrigation was not needed in 1995.

All samples were collected from the center two rows. In 1982 and 1983 at seed maturity, ten plants were randomly selected to determine seed size across main axis node position. Additionally, two-seeded and three-seeded pods were kept separate to determine seed size according to position of seeds in pods at six different node positions (3, 6, 9, 12, 15 & 18). In 1995, during seed growth, three-seeded pods from four different plants at node positions 4, 8 & 12 were sampled to determine seed growth rate and effective filling period of seeds according to the position in pods. Cotyledon cell numbers, cell growth rates and cell weights were also determined.

Cotyledon Cell Number

In 1995 the number of cotyledon cells was estimated using the method described by Swank et al. (1987). Seeds were dried for 24 hours (at about 70°C) for dry weight determination and then were allowed to imbibe water for 8-10 hours, after which the seed coat and embryo axis were removed. Cotyledons were placed in a fixative formalin acetic acid solution (Berlyn and Miksche, 1976). After 24 hours in formalin acetic acid, each cotyledon was cut finely and digested in 40 ml of chromic acid solution (80g chromic acid/ liter water) (Reinert and Yeoman, 1982). The chromic acid was removed after 5

days, and cotyledons were macerated before diluting with water. An aliquot of the cell suspension was placed on a hemacytometer, and the cells counted under X100 magnification. For the material not completely digested, the suspension was filtered, and the residues were dried and weighed. The number of cells counted was adjusted by the proportion of the total cotyledon mass not digested to give the total number of cells in the cotyledon. Cell growth rate was calculated by dividing the seed growth rate by the cell number. Cell volume was obtained by dividing the maximum fresh seed volume by the number of cotyledon cells. The estimated number of cells per cotyledon pair were calculated using the known volumes.

Seed Growth Rate and Effective Filling Period

Three-seeded pods at node positions 4, 8 and 12 were collected from soybean plants in 1995. After samples were collected, seed fresh mass was measured and seed volume was determined by water displacement method. Seeds were then dried (at about 70°C) for 48 hours and the dry weights recorded. Linear regression analysis was used to estimate the seed growth rates after eliminating the obvious non linear points from the initial and the final phases of seed development. At the final maturity, the final seed size was recorded. The length of the effective filling period was estimated by dividing the final seed size by the seed growth rate (Daynard et al., 1971).

Results and Discussion

In the 1982 and 1983 studies, 23 soybean genotypes belonging to three different maturity groups were evaluated for seed size variations within two-seeded and three-seeded pods at six different node positions on the main axis (Herbert and Zhi-yi, 1984). In these genotypes the size ranged from about 100 to 220 mg/seed (Figure 4.1). It was found that in all these genotypes the seed size remained relatively constant across all node positions irrespective of the number of seeds per pod (Figure 4.2). However, it was observed that the basal seed in both two-seeded and three-seeded pods was significantly smaller at maturity than the middle and terminal seed (Figure 4.3). This trend occurred regardless of genotype and was observed across node position and year, thus showing it to be under the genetic control of the plant.

In 1995, the study was undertaken to determine the effect of the seed position on the rate of seed growth and filling duration within the pod. As in 1982 and 1983, position of seed within a pod had an effect on the pattern of seed growth and final seed weight at maturity (Table 4.1). The basal seed, which was the closest to the vascular connection to the pod, lagged behind in dry matter accumulation throughout seed development. It had a significantly lower rate of seed growth (6%) as compared to the middle and terminal seed in the pod. There was no significant effect of the seed position within pod on the length of the effective filling period. Egli et al. (1978) also reported low rates of seed growth of the basal seed in pods as compared to the middle and terminal seed, with no differences in filling duration of soybean seeds.

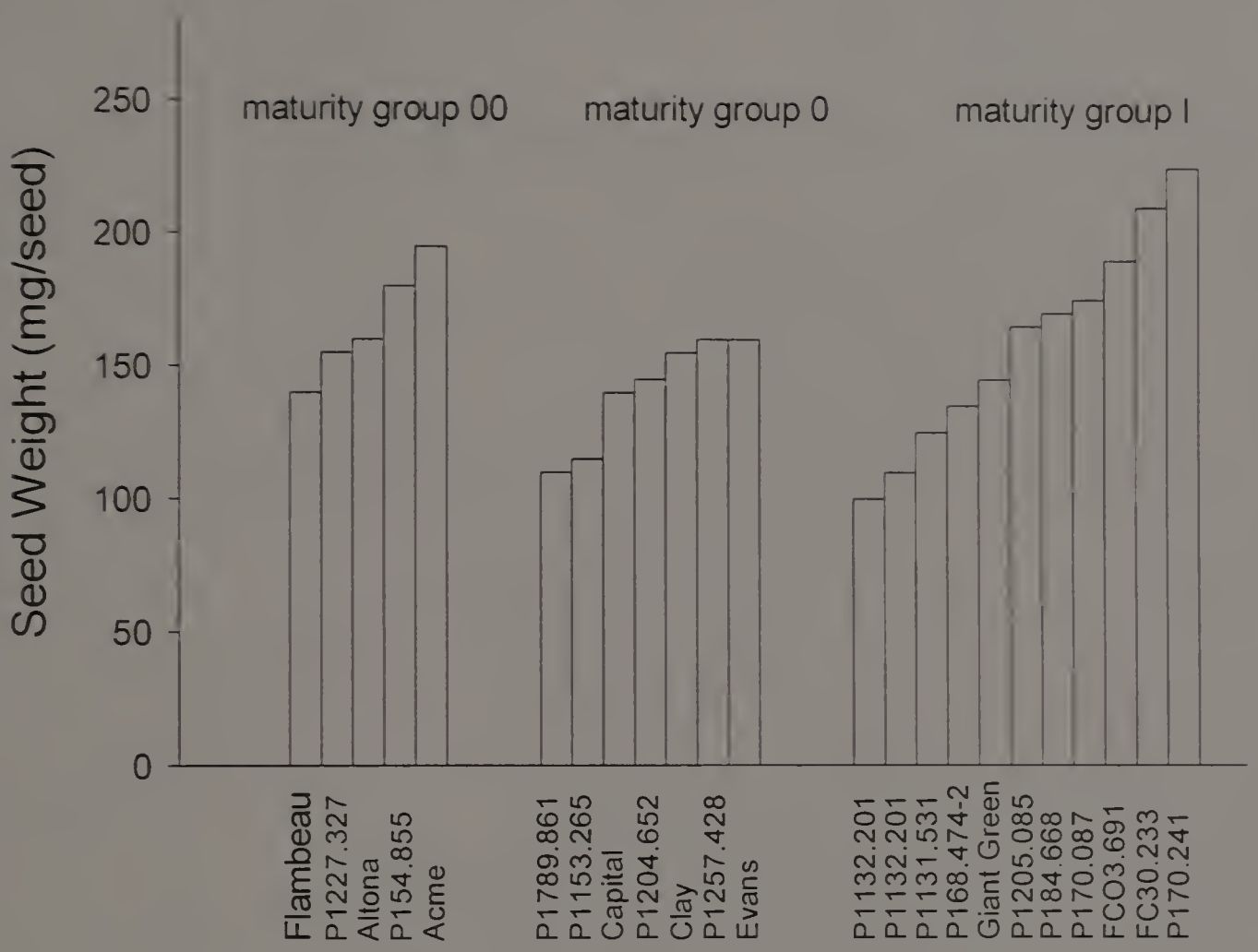

Figure 4.1 Mean weight per seed for different maturity groups of 23 genotypes. (Herbert and Zhi-yi, 1982 & 1983).

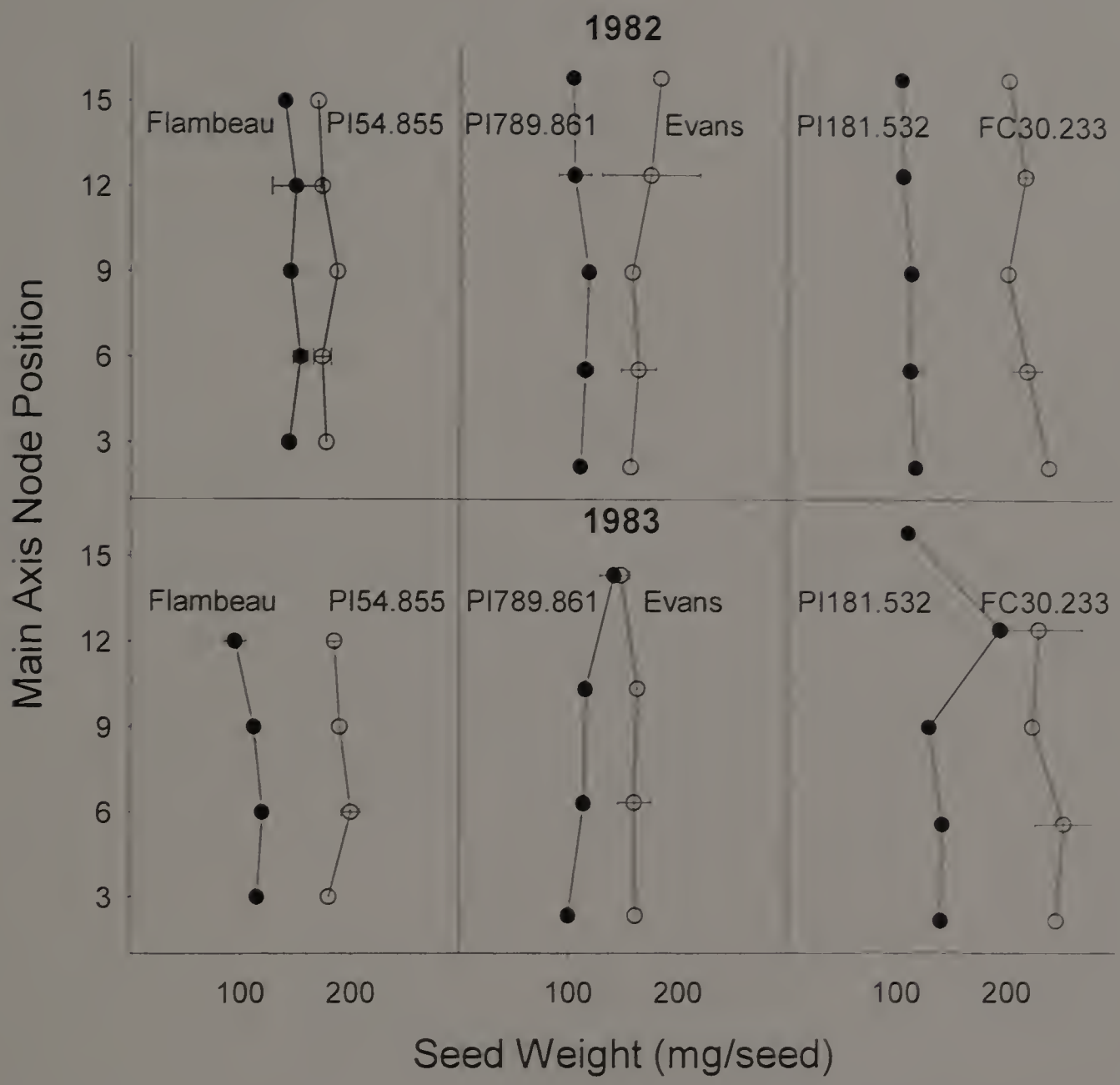

Figure 4.2 Mean weight per seed for main axis nodes of varying maturity group soybeans. Six select varieties are presented.

(Herbert and Zhi-yi, 1982 & 1983).

- The standard error of means at 95% confidence level are shown by horizontal bars.

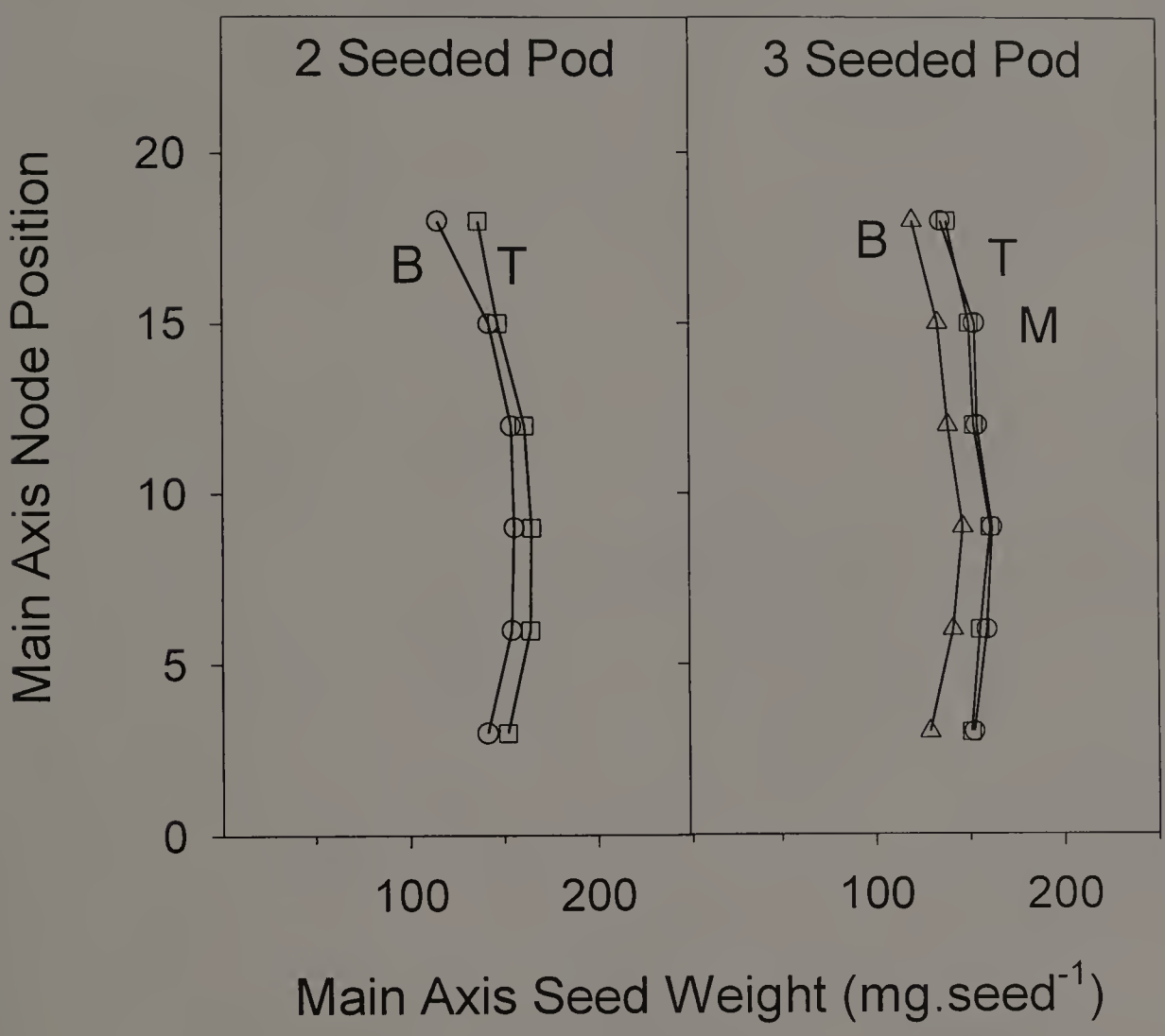

Figure 4.3 Seed dry weight for seeds within two seeded and three seeded pods averaged for 23 genotypes in 1982 and 1983.

(Herbert and Zhi-yi, 1982 & 1983).

- Basal (B), middle (M) and terminal (T)

Table 4.1 Seed growth characteristics of soybean in relation to position in three seeded pods in 1995.

Position	Final Seed Size (mg seed^{-1})	Seed Growth Rate (mg seed^{-1} day^{-1})	Effective Filling Period (days)
B	178.6 b	12.2 b	14.6 a
M	185.4 a	12.9 a	14.3 a
T	185.5 a	12.9 a	14.3 a

- Data points are the means of seeds from 4 plants and averaged over 4 replications.
- B, M and T represent basal, middle and terminal positions in pods respectively.
- Means within columns followed by the same letter are not significantly different at the 5% level of probability using Duncan's Multiple Range Test.

Cell division in seeds of most soybean genotypes is reported to terminate approximately 8-10 days after anthesis and subsequent growth occurs by cell enlargement (Davies, 1977; Dunphy et al., 1979; Egli et al., 1981). Several studies have shown that unfavorable temperatures during the endosperm cell division in wheat reduce the sink capacity by reducing the cell size with little or no effect on the cell number (Radley, 1978; Wardlaw, 1970). Such differences in growth rate of seeds have been correlated with number of cells in cotyledons suggesting that they could be the point of control (Egli et al., 1980). The factors which affect the rate and duration of cell division may ultimately determine the cell number per cotyledon and consequently seed size (when nutrients and water are not limiting).

We hypothesized that a variation in cell size (weight and volume) and/or cell number of cotyledons of soybean were mainly responsible for the differences in seed size within the pod. The cotyledon of seeds in the middle and terminal position had a significantly greater number of cells as compared to the cotyledon of basal seed (Table 4.2). Egli et al. (1981) and Guldan and Brun, (1985) have found much of the variation in the seed size in soybean genotypes, to be associated with the number of cells in the cotyledons. Weight and volume of cells in the cotyledons of seeds in our study did not differ between the three seeds within the pod. Also the growth rate per cell did not increase with an increase in seed size.

However, there are reports where differences in cell size of cotyledons also contributed to differences in seed sizes. Swank (1987) reported a

Table 4.2 Cotyledon cell characteristics of seeds of soybean in relation to position in three seeded pods in 1995.

Position	Cotyledon Cell Number ($X10^{-6}$)	Cell Growth Rate (ng $cell^{-1}$)	Cotyledon Cell Weight (ng)
B	8.01 b	1.52 a	22.23 a
M	8.35 a	1.55 a	22.2 a
T	8.43 a	1.54 a	22.0 a

- Data points are means of 3 replications.
- B, M and T represent basal, middle and terminal seeds, respectively.
- Means within columns followed by the same letter are not significantly different at the 5% level of probability using Duncan's Multiple Range Test.

variation in the size of the cotyledon cell to contribute to the variation in seed size among soybean genotypes. Hirshfield (1992) studied the effects of fertilizer treatments on soybean seeds and found this resulted in an increase in final seed yield which was associated with an increase in cell size of cotyledons rather than cell number.

Results from the 1995 experiment showed the number of cells in the cotyledon to be significantly correlated with the final seed dry weights (r = 0.78*), indicating that a variation in the number of cells in cotyledons contributed to the differences in seed weights within pods. Thus, within a single genotype in soybean, we suggest variation in seed size within a pod is influenced more by the cell number of cotyledons than cell size.

Summary

Variations in seed size within pods at different node positions on the main axis of soybean plants were significantly correlated with differences in seed growth rates and cell number of cotyledons. The basal seed which is closest to the vascular connection to the pod lagged behind in both fresh and dry matter accumulation throughout the seed development. It also showed a significantly lower number of cells in the cotyledons. These results show rates of seed growth and variation in cell number, rather than duration of seed fill and cell size, are important parameters in determining seed size in soybean. This suggests that the seed growth rate is at least partially controlled by the genetic makeup of the seed as it varied with seed position in pods, and also because

this difference was consistent across node positions, genotypes and irrespective of number of seeds in pods or pod shell dimensions.

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CHAPTER 5

SUMMARY

In 1994-1995 seasons field experiments were conducted to investigate the effects of light enrichment and shade treatments on endogenous abscisic acid (ABA) levels and seed growth characteristics of short season soybean 'Evans' genotype.

Chapter 2 examines nodal distribution of seed yield components in soybean under different light enrichment and shade treatments. Increases in seed yield was contributed largely by an increase in pod number per node in LE_1 (light enrichment - beginning flowering stage) and more than at LE_2 (beginning pod development, R_3-stage). However, at LE_2 an increase in seed size in plants was observed, probably because the sink capacity (number of pods and seeds) in LE_2 was less as compared to LE_1. Shading soybean plants (at beginning of pod development) decreased pod number per plant, seed size and the final yield as a result of decreased available light and hence photoassimilate supply. Harvest index was increased in LE_1 showing an increase in partitioning of assimilates to reproductive sinks.

In Chapter 4, seed size variation within pods at different node positions on the main axis of soybean plant was determined. The weight of the basal seed in both the two and three seeded pods was significantly lower than the other seeds within the pod. The middle and terminal seeds showed a higher rate of seed growth and a greater number of cells in the cotyledons with no

differences in the filling duration. These differences in seed size were consistent across node positions, genotypes and irrespective of number of seeds within pods, thus showing the seed size to be under the genetic control of the plant.

Chapter 3 examines the relationship between seed size, seed growth rate and changes in the endogenous ABA levels in seed components. Higher rates of seed growth and final seed size along with increased cotyledon cell number were observed with light enrichment from the beginning of pod development stage. These differences in cotyledon cell number were significantly correlated with final seed weights and seed growth rates.

Endogenous ABA levels were determined in cotyledons and seed coats of soybean. Soybean seeds showed a higher concentration of ABA in cotyledons as compared to seed coats throughout the seed development, thus indicating that ABA is not equally distributed within the seed. Endogenous ABA levels in soybean were significantly correlated with seed growth rates and cotyledon cell number. Shading resulted in lowering of endogenous ABA levels along with a reduction in seed growth rates and cotyledon cell number. Decreased light availability along with lowering of endogenous ABA levels may decrease assimilate availability during seed development, thereby regulating cotyledon cell number and seed growth rate for the remainder of seed filling period.

Variations in seed size within pods at different node positions on main axis of soybean plant were significantly correlated with the number of cells

in the cotyledons. Across 23 genotypes of soybean tested (Herbert et al., 1984) the basal seeds within the pod was smaller in final seed size in both the two and the three seeded pods. The basal seeds in the pod showed lower rate of seed growth and fewer number of cells in the cotyledon as compared to both the middle and terminal seeds. Subjecting soybean plants to light enrichment also showed higher rates of seed growth along with greater number of cells in the cotyledons compared to smaller sized seeds. Results indicate that seed growth rate and cotyledon cell number can be influenced by light availability to individual plant, this helps explains variations in seed size within a single genotype that are observed between years and locations.

Environmental factors are known to effect endogenous ABA levels in kernels of barley plants where higher ABA levels were reported under high sunlight intensities (Tietz et al., 1981) and in soybeans, where short day photoperiods resulted in an earlier accumulation of endogenous ABA and sucrose, which may have increased assimilate availability to the developing seeds(Morandi et al., 1990). Complex mechanisms are involved in partitioning of assimilates during grain filling period as different sources like leaves, green stem parts and pod walls contribute photoassimilates to developing seeds. More information is needed on the effects of changes in environmental factors like variation in light intensities, temperature and photoperiods on endogenous ABA concentrations in seed tissues and its effect on partitioning of assimilates to seeds and seed growth rates.

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