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Effects of Water Deficit on Leaf Growth during Vegetative Growth Period in Soybean

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Leaf area is critical for crop light interception, and thereby has a substantial influence on crop yield. This experiment was conducted to characterize the development of soybean [*Glycine max* (L.) Merr.] leaf area. Plastochron index and leaf relative growth rate of Jackson was contrasted with the PI416937, which also has demonstrated tolerance to drought. First, plastochron ratio (PR) and plastochron index (PI) were evaluated in greenhouse to compare the leaf growth rate between two genotypes under well-watered condition. There was reasonable constancy of PR between two genotypes. The PR means of Jackson and PI416937 were 0.41 and 0.44, respectively. A fairly smooth increase of PI during vegetative stage was observed. Second, the relative growth rates were graphed against leaf area, normalized with respect to final leaf area, under well-watered and water-deficit conditions. Leaf growth was sustained longer in well-watered condition than water-deficit condition and there was a sizable proportion of leaves which was ceased earlier their growth in water-deficit condition compared to well-watered condition. The leaf relative growth rate of Jackson until leaves had completed at 45% of their growth during water deficit period was higher than that of PI416937.

Keywords : soybean, water deficit, plastochron ratio, plastochron index, leaf relative growth rate.

The importance of crop leaf area in influencing yield has long been recognized. Simple models which examined crop yield potential have further emphasized the importance of crop leaf area development (Sinclair *et al.*, 1981; Monteith and Scott, 1982; Chae and Lee, 1988). Attempts to segregate the various components of leaf area development have been made (Hofstra *et al.*, 1977; Littleton *et al.*, 1979). Hofstra *et al.* (1977) divided the processes of leaf area development into leaf emergence rate, leaf expansion rate, and final area of individual leaves. The rate of emergence was expressed as an increase in the plastochron index (PI). PI is an integer count of the number of emerged leaves, plus a decimal fraction representing the progress of the emerging leaf toward full emergence. The next step in defining leaf area development was to determine leaf expansion rate, and either duration of leaf expansion or final leaf area. While this approach aids in understanding the physiological events determining the areas of individual leaves, it may be unnecessarily complicates in efforts to predicted cumulative plant leaf area (Sinclair, 1984). Under non-drought conditions each leaf seemingly has a well-defined growth rate which depends on temperature and final leaf area that depends on position on the plant (Hofstra *et al.*, 1977; Dennett *et al.*, 1978; Littleton *et al.*, 1979). Ogbuehi and Brandle (1981) found highly significant linear relationships for soybeans between a simple count of numbers of leaflets per plant and plant leaf area.

Soybean leaf expansion has been shown to be directly linked to leaf turgor pressure (Bunce, 1977; Wenkert *et al.*, 1978). In addition, the rate of soybean leaf emergence has been shown to be sensitive to drought (Vendleland *et al.*, 1982). Muchow (1985b) observed both effects when contrasting the drought response of two soybean cultivars. The net result of severe drought stress and reduced leaf area on the amount of intercepted radiation and soybean biomass production was demonstrated by Muchow (1985a).

The potential for using leaf observations to study growth was greatly enhanced by development of the plastochron index (PI) as proposed by Erickson and Michelini (1957). Erickson and Michelini (1957) found the leaves appeared at a uniform rate and expanded in a similar manner with time under experimental conditions.

This experiment was conducted to characterize the development of soybean leaf area. PI, PR and leaf relative growth rate of Jackson was contrasted with the PI416937, which also has demonstrated tolerance to drought.

MATERIALS AND METHODS

Cultural details

Two genotypes were evaluated for the response of their

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Table 1. Maturity groups (MG) and notable characteristics of genotypes evaluated.

Genotypes	MG	Characteristics	Reference(s)
Jackson	VII	Water-deficit tolerant	Sall and Sinclair (1991) Serraj and Sinclair (1996) Serraj *et al.* (1997)
PI416937	VI	Osmotic adjustment in roots,	Goldman *et al.* (1989)
		Aluminum tolerance,	Hudak and Patterson (1989)
		Water-deficit tolerant	Patterson and Hudak (1996)

leaf area development to water deficits. These genotypes represented putative tolerance to water deficit (Table 1). Seeds of Jackson and PI416937 were sown in November 18, 1995 in 15 cm diameter pots, with a volume of approximately 1.9 L, in a greenhouse at Fayetteville, Arkansas (latitude 36°5'N). The potting mixture was a N-free peat, perlite, and vermiculite mixture obtained from Sun Gro Horticulture Inc., 1830 Knob Hill, Garland, TX75403. The potting mixture was inoculated with *Bradyrhizobium japonicum* (USDA 110) at the time of sowing. One-half liter of full strength, nitrogen-free nutrient solution (de Silva *et al.*, 1996), pH 6.8, was added to each pot. Subsequently, 250 ml of nutrient solution was applied one time per week until the beginning of water-deficit treatment, at V6 growth stage (Fehr and Caviness, 1977). After draining overnight, pot capacity weight was determined. Plants were thinned to one plant per pot at V3 growth stage. Day-night temperatures were 30 and 22 (±2)°C, which were automatically controlled heating and cooling systems. Natural illumination was supplemented with 1000 W metal halide lamps for a day length of 15 h.

Pots were weighed at 0900 h each day, and well-watered (control) plants were maintained at 70% of their pot capacity weight by rewatering daily until initiation of water-deficit treatments. At V6 growth stage, half the plants were allowed to dry until the pot weight was 36% of the pot capacity weight (water-deficit treatment). Pots were sealed inside plastic bags to minimize evaporative losses. Plants were maintained at the target weight for an additional 14 days.

Fraction of transpirable soil water

Transpirable soil water (TSW) at pot capacity was defined as the difference between the pot capacity and the pot weight when plant transpiration was <10% of the well-watered plants (Ritchie, 1981; Sinclair and Ludlow, 1986). TSW was assumed to be zero at 29% of the pot capacity weight, based on results of Purcell and King (1996) with the same potting mixture. Therefore, the fraction of transpirable soil water (FTSW) (Ritchie, 1981) was calculated at any pot weight as:

$$FTSW=[(\text{pot weight} - (\text{pot capacity weight} \times 0.29)]/TSW \quad (1)$$

FTSW values for the control and stress treatments were 0.58 and 0.11, respectively.

Plastochron index (PI)

Plants for determination of plastochron index for this experiment were identified and tagged after the first trifoliate leaf had emerged. Four plants in each genotype were observed at semiweekly intervals under well-watered condition.

At each observation the length of the center leaflet of the uppermost fully expanded leaf and the center leaflets of all leaves above it were measured. No attempt was made to measure any leaflets shorter than 8 mm. Measurement of leaflet length was made to the nearest millimeter. Usually data were recorded on the uppermost four or five leaves per plant. Calculations of the plastochron ratio (PR) and PI for each plant were made according to the approach presented by Vendeland *et al.* (1982). The PR was calculated as the logarithm of the ratio of the leaflet lengths from the measurements on the two uppermost leaves. After establishing the value of PR, the PI was calculated only from the length of the uppermost leaf. The PI and PR were calculated as;

$$PI = n + \{[\log(Ln) - \log(R)] / [\log(Ln) - \log(Ln+1)]\} \quad (2)$$

$$PR = \log (Ln/Ln+1) \quad (3)$$

where n=number of leaves longer than reference length (R), Ln=length of leaf n (which by definition is greater than or equal to R) and $Ln+1$ = length of leaf n+1 (which by definition is less than R).

Relative leaf growth rate

Center leaflet lengths were measured every morning for the uppermost four leaves to 4 plants in each genotype and water treatment combination. Center leaflet lengths were converted to leaf areas by means of a linear regression equation (R^2=0.95**) established from 45 leaflets (Fig. 1). At the end of the experimental period, leaves that had been measured were allowed to complete growth under well-watered conditions. Upon full expansion, the leaves were measured and averages of the final areas of each genotype were used to express sampled leaf areas on a normalized basis.

Experimental design and statistical analysis

Randomized complete block design was used involving

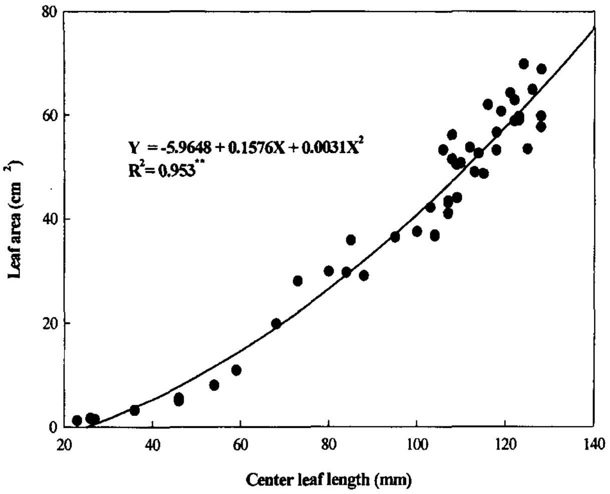

Fig. 1. Relationship between leaf area and center leaf length measured in two soybean genotypes and water treatments combined.

two water treatments, two genotypes, and four replications. Linear regression analysis was used to obtain an expression for the relative growth rate of leaf area in relation to the normalized leaf area. Statistical data analysis was performed with the SAS package.

RESULTS

Determination of plastochron ratio and index

The plastochron ratio (PR) for all observations were consistently in the range of 0.34 to 0.64 and 0.40 to 0.65 for Jackson and PI416937, respectively (Fig. 2). In both of two genotypes, younger plants tended to have larger plastochron ratios than later in their growth. This is illustrated in Fig. 2 which shows the mean PR for Jackson and PI416937 during vegetative growth period. The earliest observations yielded the highest PR and then PR decreased until the fifth leaf had emerged. Through the remainder of the growth, PR held fairly constant having a value of about 0.43 in Jackson and PI416937.

There was reasonable constancy of PR between genotypes. The median and mean PR observed for each genotype after the fifth leaf emerged are shown in Fig. 2. The PR means of Jackson and PI416937 were 0.41 and 0.44, respectively.

For convenience in calculating plastochron index (PI), the value of PR was held constant at 0.41 for Jackson and 0.44 for PI416937. While the actual value of PR varied from estimated PR, the error introduced into the estimate of PI is fairly small especially as the number of emerged leaves became large. Even if the estimated PR of each genotype is two-third

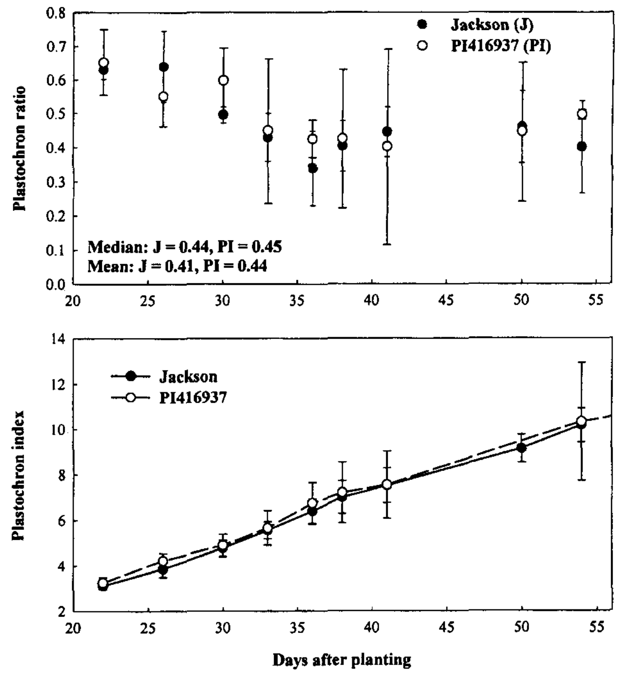

Fig. 2. Plastochron ratio and index during vegetative growth period of Jackson and PI416937.

of the actual PR, the error was essentially negligible. The relationships between two kinds of calculated PI by estimated PR and actual PR were illustrated in Fig. 3 which shows high correlation ($r=0.999^{**}$ for each genotype) between the two variables.

An important assumption in calculating PI is the reference length (R) above which a leaf is assumed to be emerging. In this study, reference length (R)=8 mm was found to be the length that most satisfactorily met the above conside-

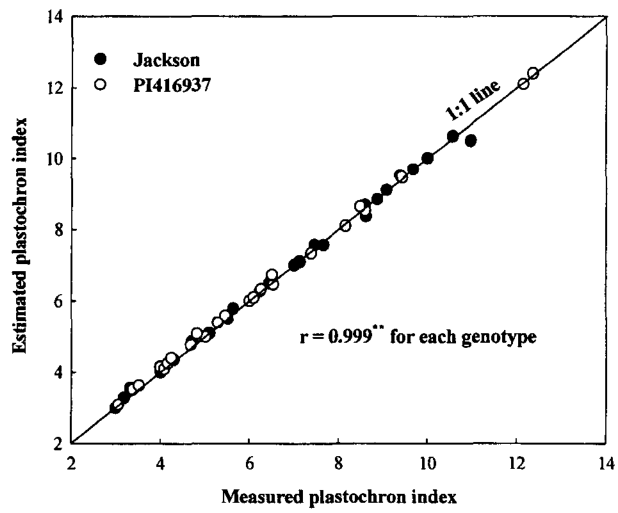

Fig. 3. Relationship between measured plastochron index in the greenhouse condition and estimated plastochron index by mean plastochron ratio for each variety.

Table 2. Response of leaf area development to water-deficit (WD) and well-watered (WW) treatments at before and after water treatment for Jackson and PI416937.

Genotype	One day before treatment		Fourteen days after treatment	
	WD	WW	WD	WW
	------------------ $cm^2 plant^{-1}$ ------------------			
Jackson	390	483	633	1013
PI416937	719	701	654	1327
$LSD_{0.05}$†	162		307	

LSD is for comparison of means within a measuring day.

rations.

Using PR=0.41 for Jackson and 0.44 for PI416937 and R=8 mm, PI was calculated at all observation dates. The calculated PI was consistent among plants within a genotype. Also, a fairly smooth increase of PI during vegetative stage was observed. The observed data for 4 plants of Jackson and PI416937 were plotted in Fig. 2.

Relative leaf growth rate

Center leaflet lengths were converted to leaf areas by means of a linear regression equation (R^2=0.95) established from 45 leaflet length and leaf area measurements (Fig. 1). There was large genotypic difference in leaf area development to water deficit. Leaf area between before and after water deficit treatment increased 62% for Jackson and decreased 9% for PI416937 (Table 2). For PI416937, the decrease in leaf area was associated with both decreased leaf production and increased leaf senescence. Relative leaf growth rates of Jackson and PI416937 in water-deficit plants were graphed with respected to their normalized leaf areas (Fig. 4). Two groups of data may be delineated in Fig. 4: small leaves to the left of the perpendicular line at 0.45 units of normalized leaf area and larger more mature leaves to the right. Realtive growth rates of leaves which had completed more than 45% of their growth were sometimes found as high as or higher than the maximum relative growth rate of young leaves which had completed less than 45% of their growth. However, there was also a sizable proportion of large leaves, the growth rate of which was as low as the growth rates of leaves which has presumably ceased cell division. Leaves which had completed less than 45% of their growth showed a regular progression of increasing relative growth rate as their normalized leaf area increased.

Relative growth rate of leaf area is equivalent to relative growth rate on a volume basis since the time intervals between successive measurements were short and changes

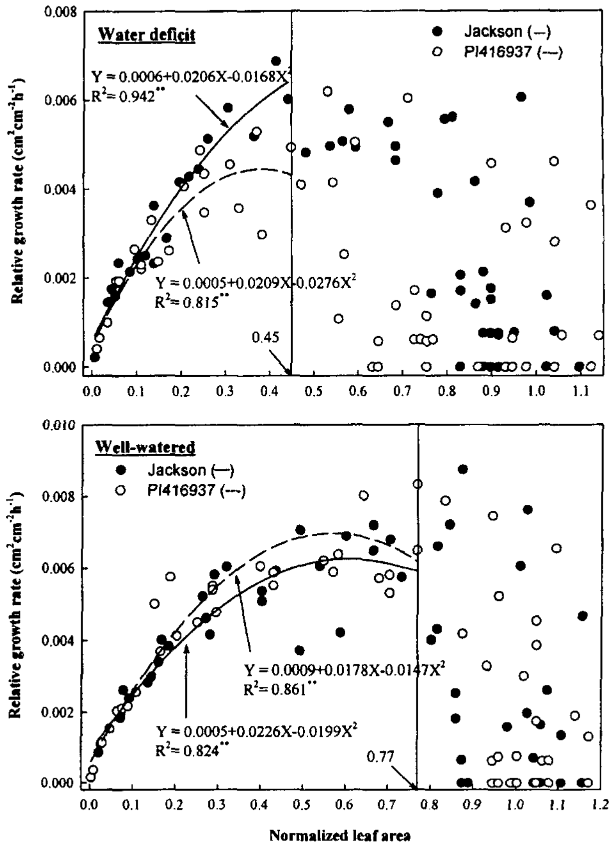

Fig. 4. Comparison of leaf relative growth rates as a function of the normalized leaf areas for Jackson and PI416937 in two different water treatments.

in leaf thickness were found to be insignificant. The relative growth rate until all leaves having reached at 45% of their final area was higher in Jackson than in PI416937.

Relative leaf growth rates of each genotype in well-watered plants were similarly graphed (Fig. 4). Even though the units dividing group were different from those in water-deficit treatment, two groups of data may be also delineated in Fig. 4: leaves to the left of the perpendicular line at 0.77 units of normalized leaf area and larger more mature leaves to the right. Leaves which had completed less than 77% of their growth showed a linear progression of increasing relative growth rate as their normalized leaf area increased. This fact indicated that the relative leaf growth rates were more kept linearly progression in well-watered condition than in water-deficit condition.

The maximum relative growth rates of leaves which had reached less than 77% of their final leaf area were higher in the well-watered treatments than in the water-deficit treatments. The relative growth rate until all leaves having at 77% of their final leaf area was higher in PI416937 than in Jackson, which was different with the case in water-deficit treatment.

DISCUSSION

Tolerance to water deficits for Jackson (Sall and Sinclair, 1991; Serraj and Sinclair, 1996) was confirmed in comparisons with PI416937. In order to characterize the development of soybean leaf area, plastochron ratio and relative leaf growth rate were evaluated. There was reasonable constancy of PR between Jackson and PI416937 under well-watered condition. The PRs of Jackson and PI416937 were 0.41 and 0.44, respectively (Fig. 2). This result corresponds closely with the PR of 0.42 observed by Sinclair (1984) in the soybean cultivar Guelph, and 0.43 observed by Vendeland *et al.* (1982) in the soybean cultivar Wilkin. Also, fairly smooth and similar progression in PI increase was observed in both genotypes (Fig. 2). These plastochron data indicated that the progression of leaf area development for both genotypes under well-watered condition was similar. Similar pattern of PR and PI in both genotypes which corresponds with leaf relative growth rates under well-watered condition were found (Fig. 4). However, the response of relative growth rates of leaves to water-deficit and well-watered conditions was different. Leaf growth is sustained longer in well-watered condition than water-deficit condition and there was a sizable proportion of leaves which was ceased earlier their growth in water-deficit condition compared to well-watered condition (Fig. 4). The relative leaf growth rate of Jackson until leaves had completed at 45% of their growth under water-deficit condition was higher than that of PI416937 (Fig. 4). In conclusion, the results of this study indicate that water deficit had virtually influence on the leaf growth rates observed in the small leaves.

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