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RESEARCH

Evaluation of Soybean Greenness from Ground and Aerial Platforms and the Association with Leaf Nitrogen Concentration in Response to Drought

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ABSTRACT

Early senescence has been noted for soybean (*Glycine max* [L.] Merr) exposed to drought. This research quantified the greenness of soybean canopy using digital image analysis throughout seedfilling for drought and well-irrigated treatments. Five genotypes ranging from maturity groups (MGs) 2 to 5 were sown in field experiments for 3 yr. Leaf nitrogen (N) concentration decreased steadily throughout seedfill for all genotypes. The Dark Green Color Index (DGCI), which was determined from pictures taken from the ground, also decreased throughout the seedfill period for all genotypes, but did not indicate treatment differences between drought and well-irrigated treatments. Aerial photographs taken from a height of 50 to 75 m also showed a progressive decline in DGCI values for all genotypes during seedfill, and also indicated that DGCI decreased faster and/or earlier in the season for the drought treatment than for the well-irrigated treatment. The results demonstrated that aerial DGCI measurements have the promise to detect early senescence associated with drought, which may have applications in evaluating differences in sensitivity to drought among soybean genotypes.

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Abbreviations: ANCOVA, analysis of covariance; DAR5, days after R5; DGCI, Dark Green Color Index; DR, drought; G_DGCI, Ground Dark Green Color Index; HSB, hue, saturation, brightness; IRR, well-irrigated; MG, maturity group; NDVI, normalized difference vegetation index; NS, nonsignificant; R1, first flower; R5, beginning of seed till; RGB: red, green, blue.

Soybean (*Glycine max* L. [Merr.]) grain is composed of approximately 35 to 40% protein, and thus, nitrogen (N) is a major component of the seeds. In the absence of soil N, soybean plants obtain N through a combination of uptake of inorganic N from the soil and biological di-nitrogen (N_2) fixation mediated by *Bradyrhizobium japonicum* bacteria living in soybean root nodules. For soils with little available N, biological N_2–fixation contributes up to 85% of the N in the soybean plant (Mastrodomenico and Purcell, 2012).

During seedfill, N is remobilized from soybean leaves to seeds (Sinclair and de Wit, 1976; Mastrodomenico and Purcell, 2012), resulting in decreased chlorophyll and leaf N concentration, and leaf yellowing. Thus, leaf color can be used as a metric of N status of plants (Rorie et al., 2010, 2011).

Nitrogen fixation in soybean is particularly sensitive to drought (Serraj et al., 1999), and when exposed to drought during seedfill, N_2 fixation is decreased and unable to recover (Mastrodomenico and Purcell, 2013). Decreased N_2 fixation due to drought during seedfill exacerbates the remobilization of N from vegetative tissues to seeds (Korte et al., 1983; Salado-Navarro et al., 1985; Specht et al., 1986) resulting in a shortened seedfill period,

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premature senescence, and decreased yield (De Souza et al., 1997; Hoyos-Villegas et al., 2014; Salado-Navarro et al., 1985).

Numerous vegetation indices have been associated with crop chlorophyll or color (Gitelson, 2018; Kovar et al., 2019; Maimaitijiang et al., 2019; Miller et al., 2018; Richetti et al., 2019; Seo et al., 2019). For example, the normalized difference vegetation index (NDVI) is a standardized index detecting greenness of plants by contrasting the chlorophyll pigment absorption in red bands and high reflectivity of the plant materials in near-infrared bands (Gago et al., 2015; Gitelson, 2018; Richetti et al., 2019; Seo et al., 2019). Yu et al. (2016) used machine learning to build a model for predicting soybean maturity with over 93% accuracy based on NDVI values collected throughout the season using an unmanned aerial system (i.e., drone). The normalized difference red edge (NDRE) index is another standardized index, substituting NDVI's red band with red edge (Gago et al., 2015). Photochemical reflectance index (PRI) is a measurement of canopy reflectance, and detects the changes in carotenoid pigment in live foliage, indicating photosynthetic light use efficiency (Kovar et al., 2019).

The Dark Green Color Index (DGCI) is also a type of vegetation index expressing the intensity of the leaf greenness. Karcher and Richardson (2003) were the first to report use of DGCI and defined it from values of hue, saturation, and brightness (HSB) that can be obtained from standard digital photographs. Hue refers to an angle on a continuous circular scale from 0 to 360°, saturation represents the purity of the color from 0% (gray) to 100% (fully saturated color), and brightness is the relative lightness or darkness of the color from 0% (black) to 100% (white; Karcher and Richardson, 2003).

$$\mathrm{DCGI} = \left[\left(H-60\right)/60+\left(1-S\right)+\left(1-B\right)\right]/3 \quad [1]$$

Values of DGCI range from 0 to 1, corresponding to light yellow and dark green, respectively. Rorie et al. (2010, 2011) standardized DGCI measurements by including yellow and green color standards in each image, which allowed correction in DGCI values for differences in lighting conditions or cameras. From field experiments with various N fertilizer treatments in corn (*Zea mays*. L.), DGCI values of excised leaves were closely associated with N concentration (Rhezali et al., 2018; Rorie et al., 2010).

Ground imaging methods are time-consuming, not suitable for measuring large number of samples, and are typically limited to relatively small imaging areas. Remote sensing in agriculture overcomes many of the shortcomings of ground imaging methods (Gago et al., 2015; Gitelson, 2018; Maimaitijiang et al., 2019; Richetti et al., 2019; Seo et al., 2019). In this study, an aerial photographic method for measuring DGCI was evaluated and compared with measurements made from the ground. The hypothesis of this research was that under drought, DGCI would decrease faster than under well-irrigated conditions as N was remobilized to seed. The objective was to determine the response of DGCI during seedfill in response to drought for measurements made at both the ground level and from an aerial platform.

MATERIALS AND METHODS

A field experiment was conducted at the Main Experiment Station in Fayetteville, Arkansas (36°05′ N, 94°10′ W) on a Captina silt loam soil (Fine-silty, siliceous, active, mesic Typic Fragiudults) during the summers of 2012, 2013, and 2014. This experiment was divided into well-irrigated (IRR) and drought (DR) experiments that were grown side by side. Four different soybean maturity groups (MGs) from MG 2 to MG 5 were selected for evaluation of IRR and DR experiments. The soybean genotypes were AG24–30, S25-T8, S33-K5, P94Y40, and P95Y50 in 2012, S25-E5, S35-C3, P93Y72, P94Y40, and P95Y50 in 2013, and S25-E5, S35-A5, R2 36X82N, P46T21R, and AG5532 in 2014. Genotypes differed among years due to the rapid turnover of cultivars by seed companies. The purpose of including different genotypes was primarily to provide a wide range of maturity and crop development stages during the season, and to increase the probability that drought would affect genotypes of different maturity at some point during seedfill.

The experiment was conducted using a randomized complete block design with five replications. Soybean was planted on 2 June 2012, 8 June 2013, and 17 June 2014. Plots consisted of four rows 6.1 m in length and 0.46 m between rows. The seeding density was 30 seed m^{-2}. An overhead sprinkler irrigation system was installed for both IRR and DR sections of the fields. Irrigation was applied to the IRR portion when the estimated soil-water deficit (Purcell et al., 2007) reached 30 mm. The drought portion of the field was kept fully irrigated until canopy closure (approximately 6 wk after emergence) and then received irrigation approximately every third time the IRR portion received water. A total of 10 rain gauges were placed in the field (five in IRR treatment and five in DR treatment) to record the irrigation amount and rainfall. The total irrigation amount per rain gauge for each water treatment for the growing season was calculated based on the rain gauge amounts. The percent of deficit for individual water treatment was calculated using Eq. [2]:

$$\text{Deficit (\%)} = \left(1-\frac{\text{Irrigation} + \text{Rain for deficit treatments}}{\text{Irrigation} + \text{Rain for fully irrigated treatments}}\right)100 \quad [2]$$

Soybean phenological development was also recorded twice a week for each variety after first flower (R1) using the staging method of Fehr and Caviness (1977).

The greenness of the canopy for all genotypes was determined once a week after the MG 2 cultivar reached beginning of seed till (R5) by taking digital color pictures of the canopy at the top of the plants for each plot. A pink board (1.2 m by 0.6 m), with both yellow and green disks (11-cm diameter) that served as internal standards to correct for differences in lighting conditions (Rorie et al., 2010), was positioned vertically at about one-third of the plot length, and a picture was taken against the pink board from the other end of the plot across the top of the canopy. The pictures were usually taken between 1000 and 1400 h on sunny days to minimize shadows. Known Munsell color values for green and yellow disks were 6.7GY 4.2/4.1 and 5Y 8/11.1, and the corresponding DGCI values were 0.5722 and 0.0733, respectively (Rorie et al., 2010, 2011). A Canon Power Shot S5 IS camera with a resolution of 3264 by 2448 (Canon U.S.A., Inc. Lake Success, NY), was used for taking ground images. The camera had an f-stop of 1/4, a focal length of 6 mm, and an ISO of 80 with no flash. The images were saved as Joint Photographic Experts Group (JPEG) files with dimensions of 640 by 480. Images were analyzed by Sigma Scan Pro (v5.0 SPSS, Inc., Chicago, IL) for DGCI with hue values ranging from 30 to 115, and saturation values ranging from 0 to 100. A macro working with Sigma Scan Pro allowed batch analysis for determining DGCI values using the given ranges of hue and saturation (Karcher and Richardson, 2005). On the same day ground images were taken, the topmost fully-expanded leaf was sampled from three different plants in each plot for N analysis using the Dumas method with a Leco FP-428 Determinator (Leco, St. Joseph, MO) at the Soil Testing and Plant Analysis Laboratory at the University of Arkansas.

Table 1. Monthly averages of maximum and minimum temperature (T_{max}, T_{min}), rainfall, and solar radiation from June through September for 2012 to 2014 versus 30-yr average values from 1981 to 2010 (NCDC, 2016).

Year	Month	T_{max}	T_{min}	Rainfall	Solar Radiation
		—— °C	——	mm	MJ m^{-2} d^{-1}
2012	June	31.8	19.0	58	23.8
	July	35.6	22.3	62	23.7
	August	32.4	20.1	101	20.8
	September	28.0	16.7	56	16.7
2013	June	29.8	19.6	32	21.4
	July	31.6	19.9	94	22.3
	August	30.5	20.2	138	18.8
	September	29.4	17.0	92	17.7
2014	June	28.4	20.4	102	18.6
	July	29.2	18.6	37	21.1
	August	32.1	20.5	70	20.3
	September	27.3	16.0	101	16.8
30-yr average (1981– 2010)	June	28.7	16.8	127	23.0
	July	31.4	19.2	88	22.8
	August	31.7	18.4	82	21.8
	September	26.9	13.7	122	18.4

Table 2. Irrigation amounts, rainfall, and estimated deficit irrigation amounts for different water treatments from 2012 through 2014.

	Irrigation Amount/Rain Gauge			Deficit	
Year	IRR†	DR	Rainfall	IRR	DR
	——	mm	——	—— %	——
2012	443	224	276	0	30
2013	322	177	335	0	22
2014	228	102	237	0	27

† IRR, well-irrigated; DR, drought.

Ground DGCI (G_DGCI) measurements were made 10 times in 2012 and 2013 and eight times in 2014. Leaf samples were collected on nine of these dates in 2012 and on all of these same dates in 2013 and 2014. Aerial DGCI measurements were made on six (2012), six (2013), and eight (2014) of the same measurement dates as were G_DGCI measurements.

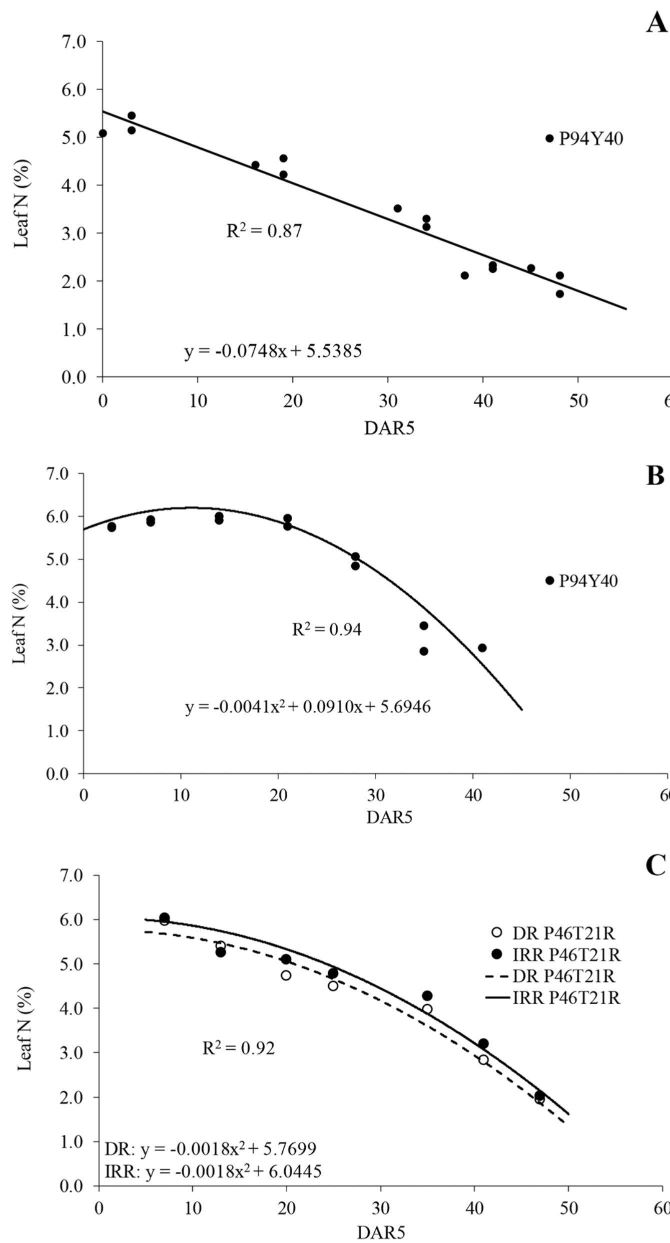

Fig. 1. Leaf N concentration versus days after Reproductive Stage 5 (DAR5). In 2012, data are presented across water treatments (not significant). Genotype was significant, and P94Y40 was used to represent the response of leaf N concentration to DAR5 similar to other genotypes (not included in the figure; A). In 2013, data are presented across water treatments (not significant). Genotype interacted with DAR5 and DAR5 × DAR5. P94Y40 was used to represent the response of leaf N concentration to DAR5 similar to other genotypes (not included in the figure; B). In 2014, a common quadratic slope was estimated for each genotype under both water treatments, but intercepts were different. Genotype P46T21R was used to represent the response of leaf N concentration to DAR5 similar to other genotypes (not included in the figure; C).

Aerial images were taken from heights of 50 to 75 m by mounting a digital camera on balloon or kite platforms. Two boards (1.2 by 2.4 m) painted with a pink background and yellow and green internal standards (~1-m diameter) were positioned on one side of the field. The balloon (approximately 1-m diameter, Southern Balloon Distributors, Miami, FL) was inflated with helium and was tethered by three strings fixed onto the balloon, and each of the strings was attached to a winding mechanism. The balloon was used as the aerial platform on calm days. A parafoil kite (2 m^2 in area, Peter Lynn Kiteboarding, The Hague, the Netherlands) was used when wind speeds were greater than 8.9 m s^{-1}. A Levitation Delta kite with a 2.75 m wing span (IntoTheWind, Boulder, CO) was used at intermediate winds ranging from 1.7 to 8.9 m s^{-1}.

A GoPro camera (Hero, DCIM/100GOPRO, GoPro, San Mateo, CA) with an f-stop of 1/3.6 and a focal length of 5 mm was used for the aerial images in 2012. The GoPro camera was

Table 3. Analysis of covariance (ANCOVA) for leaf N concentration versus days after Reproductive Stage 5 (DAR5) in 2012, 2013, and 2014. Factors considered include irrigation (IRR), genotype (Geno), DAR5, DAR5 × DAR5, and all two- and three-way interactions. Letters a, b and c represented the quadratic and linear slopes and intercept for each genotype across water treatments in the model. Nonsignificant interactions were removed from the model stepwise.

Leaf N 2012

Source	DF†	Mean Square	F Value	Pr > F	Adj. R^2
IRR	1	0.0355	0.16	0.6917	0.87
Geno	4	0.9099	4.08	0.0061	
DAR5	1	61.0129	273.40	<0.0001	
DAR5 × Geno	4	0.8615	3.86	0.0082	

y = bx + c

Geno	Relative MG	Irri	b	c
AG24–30	2.4	DR/IRR	−0.0901	5.3234
S25-T8	2.5	DR/IRR	−0.0938	5.3048
S33-K5	3.3	DR/IRR	−0.0516	4.7331
P94Y40	4.4	DR/IRR	−0.0748	5.5385
P95Y50	5.5	DR/IRR	−0.0567	4.4581

Leaf N 2013

Source	DF	Mean Square	F Value	Pr > F	Adj. R^2
IRR	1	0.0443	0.33	0.5662	0.94
Geno	4	0.1583	1.19	0.3259	
DAR5 × Geno	5	0.4759	3.58	0.0072	
DAR5 × DAR5	1	7.8263	58.8	<0.0001	
DAR5 × DAR5 × Geno	4	0.9263	6.96	0.0001	

y = ax^2 + bx + c

Geno	Relative MG	Irri	a	b	c
S25-E5	2.5	DR/IRR	−0.0025	0.0243	5.6946
S35-C3	3.5	DR/IRR	−0.0026	0.0275	5.6946
P93Y72	3.7	DR/IRR	−0.0022	0.0075	5.6946
P94Y40	4.4	DR/IRR	−0.0041	0.091	5.6946
P95Y50	5.5	DR/IRR	−0.0001	−0.0629	5.6946

Leaf N 2014

Source	DF	Mean Square	F Value	Pr > F	Adj. R^2
IRR	1	1.3507	8.74	0.0043	0.92
Geno	4	1.1057	7.15	<0.0001	
DAR5 × DAR5	1	112.6347	728.55	<0.0001	

y = ax^2 + c

Geno	Relative MG	Irri	a	c
S25-E5	2.5	DR	−0.0018	5.6197
		IRR	−0.0018	5.8942
S35-A5	3.5	DR	−0.0018	5.4464
		IRR	−0.0018	5.7209
R2 36X82N	3.6	DR	−0.0018	5.5603
		IRR	−0.0018	5.8349
P46T21R	4.6	DR	−0.0018	5.7699
		IRR	−0.0018	6.0445
AG5532	5.5	DR	−0.0018	4.9866
		IRR	−0.0018	5.2612

† DF, degrees of freedom; MG, maturity group; DR, drought; IRR, well-irrigated.

set to take photos every 2 s. The original GoPro lens (2.5 mm) was replaced with a lens with a narrower field of view (6 mm) to lessen the "fisheye" distortion (RageCams, Sparta, MI). A Canon PowerShot S100 camera with an f-stop of 1/4 and a maximum focal length of 24 mm was used in 2013 and 2014, which ensured less distortion. The images were saved as JPEG files with dimensions of 1600 by 1064. Using this camera, three images were taken in a sequence at three different exposures. An intervalometer was installed on the camera's SD memory card from the Canon Hack Development Kit (CHDK, www.chdk.fandom.com/wiki/CHDK), which allowed the sequence of three pictures to be taken continuously at 2 s intervals. The camera was suspended from one of the balloon tether lines or from the kite line using a picavet, which dampened the movement of the camera while suspended.

After the kite or balloon with the camera was lifted about 5 m, the picavet was attached to the string of the kite or one string of the balloon, and the camera was turned on. Then the aerial platform was lifted to a height that allowed the entire width of the field to be captured. After the camera was centered above the field, the balloon or kite system was walked slowly through the field. Color digital images were then processed using GIMP 2.8 (www.gimp.org, accessed 13 September 2019) to obtain the RGB values for individual plots. The RGB values were converted to HSB values using an online RGB to HSB convertor (http://www.rags-int-inc.com/PhotoTechStuff/AcrCalibration/RGB2HSB.html, accessed 13 September 2019), and HSB values were then used to calculate DGCI values. The DGCI values were corrected using the yellow and green disks as internal standards, assuming there was a simple linear response between known DGCI values of the internal standards and observed DGCI values (Rorie et al., 2011).

Statistical Analysis

Leaf N was regressed against days after R5 (DAR5) using analysis of covariance (ANCOVA) to fit a quadratic model in which the coefficients were allowed to vary depending on irrigation and genotype. To wit, using ANCOVA we were able to make direct comparisons of genotype and irrigation-treatment effects on leaf N while considering DAR5 as a continuous variable. Using the methods of Milliken and Johnson (2002), ANCOVA for leaf N versus DAR5 was performed using a general linear model (SAS, v. 9.2, SAS Institute, Cary, NC) in which replication was considered random and genotype and irrigation were considered fixed. The model for leaf N was initially fit by including the class variables (genotype and irrigation), linear and quadratic terms for DAR5, and all possible two- and three-way interactions of the class variables with linear and quadratic terms. The initial model was re-evaluated by removing nonsignificant ($P > 0.05$) terms from the model one at a time from the highest to lowest order interactions, but keeping the main factors of irrigation and genotype. The whole models for G_DGCI vs. leaf N, G_DGCI vs. DAR5 and aerial DGCI vs. DAR5 were similar to the ANCOVA of leaf N vs. DAR5, with similar rules for removing nonsignificant factors.

Determination and presentation of an appropriate regression model for these variables depended on the ANCOVA

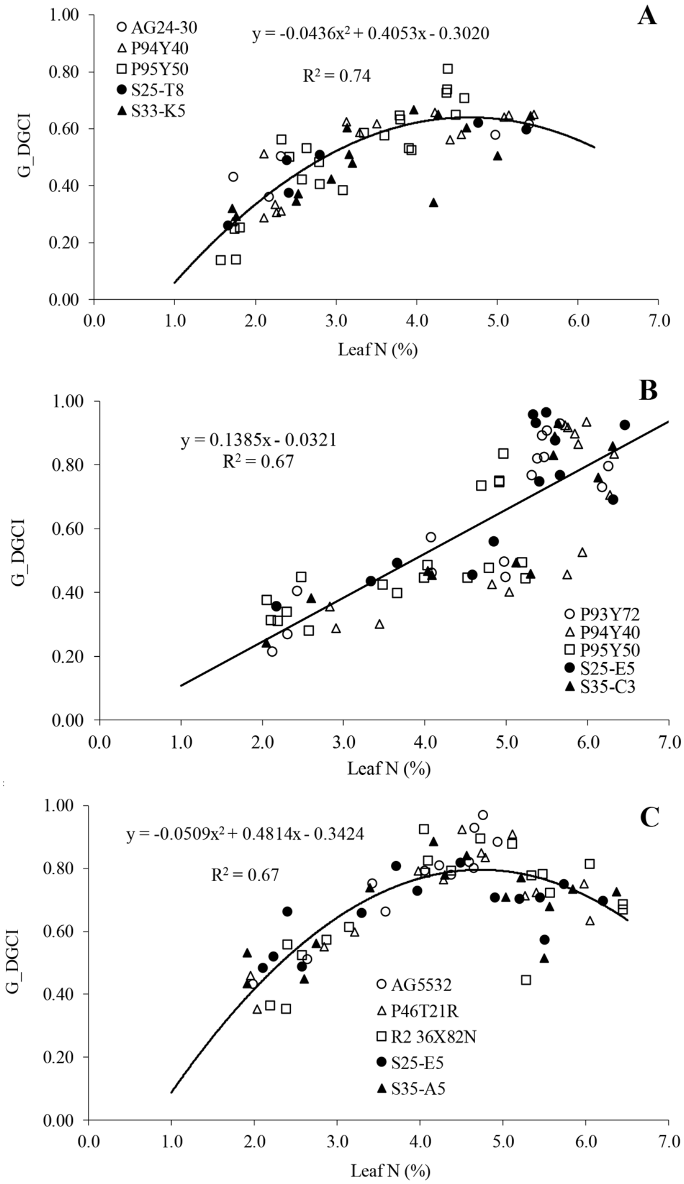

Fig. 2. Ground dark green color index (G_DGCI) versus leaf N concentration across genotypes and water treatments (genotype × water treatment interaction was not significant) for 2012 (A), 2013 (B), and 2014 (C).

results (Milliken and Johnson, 2002). For example, if both linear and quadratic terms were significant ($P \leq 0.05$), the regression equation took the form of $y = ax^2 + bx + c$, but if the linear term was not significant and the quadratic term was significant, the linear term was removed from the model (i.e., $y = ax^2 + c$). Likewise, if the genotype factor was significant in the model, separate coefficients were predicted for individual genotypes, but if genotype was not significant, data from all genotypes were used to predict the regression equation. A similar approach was used to determine whether separate regression equations should be made for DR and IRR treatments.

RESULTS

During the growing season, the average maximum temperature in 2012 was greater than 30°C from June through August, whereas the average maximum temperature exceeded 30°C for July and August in 2013, and for August in 2014 (Table 1). Similarly, solar radiation was higher in 2012 than the other 2 yr, and similar to the 30-yr average (Table 1). Though 2014 had the least precipitation for the growing season, the average maximum temperature in 2014 was less than those observed in 2012. The estimated deficit irrigation amounts were greatest for 2012 (30%), least for 2013 (22%), and intermediate for 2014 (27%; Table 2).

Leaf N versus DAR5

Leaf N concentration decreased with increasing DAR5 in all years (Fig. 1a), with ANCOVA accounting for between 87 and 94% of the variation (Table 3). In 2012, leaf N concentration decreased linearly and was not affected by irrigation, but the rate of decrease differed among genotypes (Table 3). The linear coefficient ranged between −0.0516 (S33-K5) and −0.0938 (S25-T8) with intercept values ranging between 4.4581 and (S33-K5) and 5.5385 (P94Y40). There was a tendency for the linear coefficient to increase and the constant to decrease as relative maturity increased. Based on the ANCOVA, each genotype responded differently but had similar responses for both DR and IRR treatments, which, if placed in the same figure, would require different symbols and lines for all five genotypes. Therefore, P94Y40 was chosen to represent the general response of leaf N concentration to DAR5 in 2012 (Fig. 1a). The regression coefficients for all genotypes are in Table 3.

In 2013, both the quadratic and linear effects were significant for the decrease in leaf N concentration, which differed among genotypes but was similar between irrigation treatments (Table 3). The quadratic coefficient ranged from −0.0001 (P95Y50) to −0.0041 (P94Y40), and the linear coefficient ranged from −0.0629 (P95Y50) to 0.091 (P94Y40). The absolute magnitude of the quadratic coefficient for P94Y40 resulted in the most curvature whereas the absolute magnitude of the quadratic coefficient for P95Y50 resulted in a response that was essentially a straight line. For simplicity, the response of P94Y40 is shown in Fig. 1b and coefficients for all genotypes are given in Table 3.

In 2014, the quadratic term was significant, but the quadratic term did not interact with genotype (Table 3). The main effects of genotype and irrigation were significant, but the linear term for DAR5 was not significant. Therefore, there was a common quadratic coefficient for all genotypes under DR and IRR conditions, but there were different intercepts for all genotypes with the IRR intercept within a genotype being greater than the DR intercept. Thus, within a genotype, the DR treatment senesced (i.e., yellowed) prior to the IRR treatment. The general response for P46T21R is shown in Fig. 1c, and coefficients for all genotypes are in Table 3.

DGCI versus Leaf N

For all 3 yr, G_DGCI increased with increasing leaf N concentration (Fig. 2a–2c) with ANCOVA accounting for between 67 and 74% of all variation (Table 4). Likewise, for all 3 yr, G_DGCI was not affected by either irrigation or genotype. For 2012 and 2014, G_DGCI values increased with increasing leaf N concentration between 1.5% and 4.5% (Fig. 2a, Fig. 2c, respectively); at leaf N concentrations above 4.5%,

Table 4. Analysis of covariance (ANCOVA) for ground dark green color index (G_DGCI) versus leaf N concentration in 2012, 2013, and 2014. Factors considered include irrigation (IRR), genotype (Geno), DAR5, DAR5 × DAR5, and all two- and three-way interactions. Letters a, b, and c represented the quadratic and linear slopes and intercept for each genotype across water treatments in the model. Nonsignificant interactions were removed from the model stepwise.

Source	DF†	Mean Square	F Value	Pr > F	Adj. R^2
G_DGCI 2012					
IRR	1	0.0092	1.30	0.2589	0.74
Geno	4	0.0062	0.87	0.4860	
Leaf_N	1	0.2554	36.14	<0.0001	
Leaf_N × leaf_N	1	0.1438	20.35	<0.0001	
G_DGCI 2013					
IRR	1	0.0019	0.10	0.7520	0.67
Geno	4	0.0206	1.07	0.3791	
Leaf_N	1	2.0521	106.27	<0.0001	
G_DGCI 2014					
IRR	1	0.0003	0.03	0.8583	0.67
Geno	4	0.0046	0.54	0.7094	
Leaf_N	1	0.6103	71.26	<0.0001	
Leaf_N × leaf_N	1	0.4557	53.21	<0.0001	

† DF, degrees of freedom.

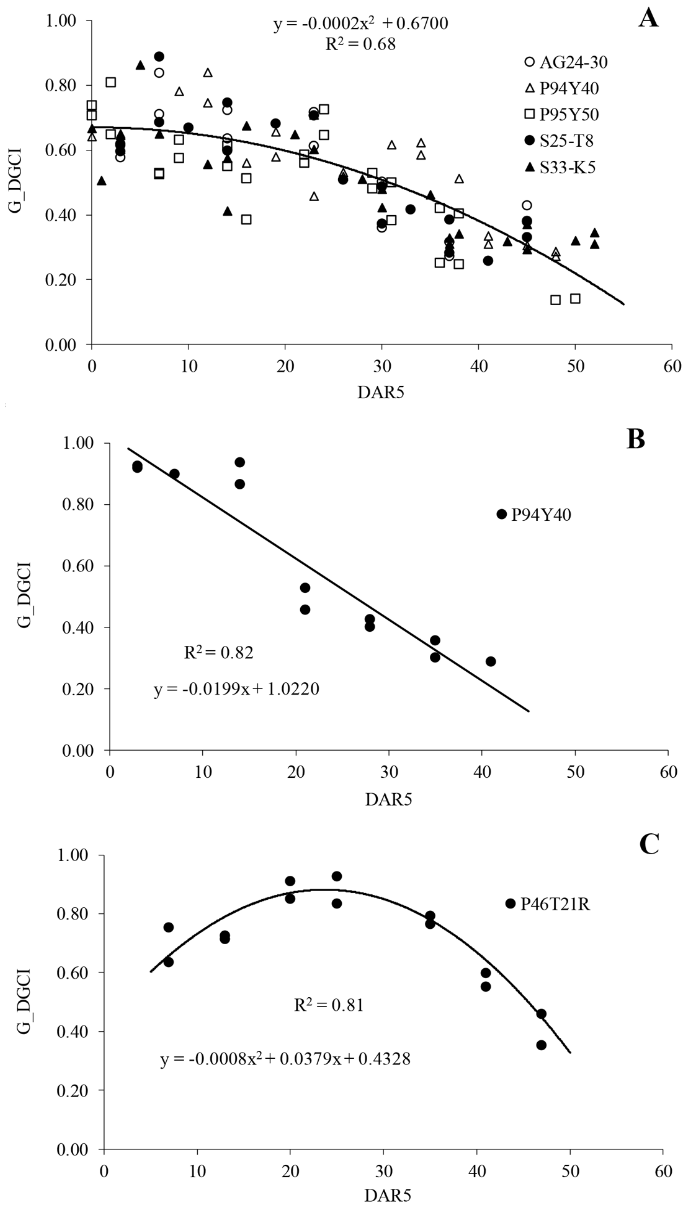

Fig. 3. Ground dark green color index (G_DGCI) versus days after R5 (DAR5) across genotypes and water treatments (genotype × water treatment interaction was not significant) in 2012 (A). G_DGCI versus DAR5 across water treatments (not significant) in 2013 (B) and in 2014 (C). Genotype P94Y40 and P46T21R were used to represent the response of G_DGCI to DAR5 in 2013 and 2014, respectively, which was similar to other genotypes (not included in the figure).

there was a slight decrease in G_DGCI. In contrast, in 2013, G_DGCI increased linearly with increasing leaf N concentration ranging from 2% to 6.5% (Fig. 2b). Because there was no significant effect of genotype or irrigation for any of the years (Table 4), all the data within a year are presented in the same figure with the same regression model.

The relationship of aerial DGCI to leaf N concentration was similar to the response of G_DGCI to leaf N concentration (data not shown). There was a curvilinear increase in aerial DGCI as leaf N increased, reaching a plateau between 4 and 5% N. In 2012, there were insufficient data to perform ANCOVA, but in 2013 and 2014, ANCOVA of aerial DGCI versus leaf N concentration accounted for 87 and 90% of the variation, respectively.

G_DGCI versus DAR5

G_DGCI decreased with increasing DAR5 in all years (Fig. 3a–3c) with ANCOVA accounting for between 68 and 82% of variation (Table 5). In 2012, G_DGCI decreased quadratically, and was not affected by either irrigation or genotype. Therefore, a common regression equation including all genotypes and including DR and IRR treatments is represented by a single equation (Fig. 3a).

In 2013, there was a linear decrease in G_DGCI that differed among genotypes, but was similar between irrigation treatments (Table 5). There was no discernable pattern among genotypes in the linear or intercept coefficients. Figure 3b illustrates the general response of G_DGCI vs. DAR5 for P94Y40; regression coefficients for the other genotypes are given in Table 5.

In 2014, ANCOVA indicated that G_DGCI was significantly affected by both linear and quadratic terms for DAR5 and by genotypes but not irrigation treatments (Table 5). Thus, there were separate linear, quadratic, and intercept coefficients for all genotypes that were the same for DR and IRR treatments within a genotypes. Figure 3c illustrates the representative response of G_DGCI vs. DAR5 for P46T21R in 2014. Other genotypes had similar responses, and their regression coefficients are provided in Table 5.

Aerial DGCI versus DAR5

Similar to G_DGCI, aerial DGCI decreased with increasing DAR5 in all years (Fig. 4) with ANCOVA accounting for between 84 and 89% of the variation (Table 6). In 2012, aerial DGCI decreased quadratically, and the rate of decrease differed among genotypes and between irrigation treatments, but within a genotype the linear and quadratic coefficients were the same (Fig. 4a, Table 6). The intercept values for the IRR treatment were greater than for the DR treatment for all genotypes, indicating that yellowing occurred earlier for the DR treatment. There was more curvature in the response of MG 2 genotypes compared with later-maturing genotypes (i.e., more negative quadratic coefficient, Table 6). The quadratic coefficient for MG 4 and 5 genotypes was essentially zero, resulting in a linear DGCI decrease with DAR5. It was difficult to discern the 10 possible interactions between genotype and irrigation in one graph; therefore, the response of P94Y40 was chosen to illustrate the general response (Fig. 4a). The coefficients for all genotypes are given in Table 6.

In 2013, a quadratic decrease in aerial DGCI differed between irrigation treatments, but was similar among genotypes (Fig. 4b, Table 6). There were no significant interactions between irrigation treatment or genotype with the linear and quadratic terms. The intercept was less for the DR treatment than the IRR treatment, indicating that the DR treatment senesced more quickly than the IRR treatment. Figure 4b shows the response of DGCI for DR and IRR treatments of all genotypes, but because the interaction between genotype and irrigation was not significant (Table 6), no attempt has been made to separate genotypes.

In 2014, aerial DGCI decreased quadratically but there was not significant interaction between the quadratic term and genotype (Table 6). Although there was not a main effect of irrigation treatment or genotype, there was a significant interaction between irrigation and DAR5. The ANCOVA indicated separate responses for irrigation treatments based on the same quadratic coefficient and intercept values, but with a greater linear coefficient for the IRR than the DR treatment (Table 6). Similar to 2012 and 2013, the predicted response of aerial DGCI indicated that senescence for the IRR treatment occurred later than for the DR treatment (Fig. 4c).

The decrease in aerial DGCI in the late reproductive stages of soybean in all years was similar to the response of leaf N concentration versus DAR5 (Fig. 1). As leaves gradually lost their greenness and began yellowing, N was being remobilized from leaves to seeds. Aerial DGCI was able to identify a significant effect of the DR treatment having lower DGCI than the IR treatment for all years, whereas this difference was not found with G_DGCI (Fig. 3). Aerial imaging had relatively high sensitivity in detecting the effect of drought compared with G_DGCI measurements.

Table 5. ANCOVA for ground DGCI (G_DGCI) versus days after R5 (DAR5) in 2012, 2013, and 2014. Factors considered include irrigation (IRR), genotype (Geno), DAR5, DAR5*DAR5, and all two- and three-way interactions. Letters a, b and c represented the quadratic and linear slopes and intercept for each genotype across water treatments in the model. Nonsignificant interactions were removed from the model stepwise.

G_DGCI 2012					
Source	**DF†**	**Mean Square**	**F Value**	**Pr > F**	**Adj. R^2**
IRR	1	0.0000	0.00	0.9891	0.68
Geno	4	0.0162	1.70	0.1556	
DAR5 ×DAR5	1	1.9584	205.60	< .0001	

G_DGCI 2013					
Source	**DF†**	**Mean Square**	**F Value**	**Pr > F**	**Adj. R^2**
IRR	1	0.0000	0.00	0.9826	0.82
Geno	4	0.1261	10.58	< .0001	
DAR5	1	2.7800	233.28	< .0001	
DAR5 × geno	4	0.0705	5.91	0.0005	
y = bx + c					
Geno	**Relative MG**	**Irri**	**b**		**c**
S25-E5	2.5	DR/IRR	−0.0169		1.0618
S35-C3	3.5	DR/IRR	−0.0161		0.9666
P93Y72	3.7	DR/IRR	-0.0143		0.9393
P94Y40	4.4	DR/IRR	−0.0199		1.0220
P95Y50	5.5	DR/IRR	-0.0072		0.6290

G_DGCI 2014					
Source	**DF†**	**Mean Square**	**F Value**	**Pr > F**	**Adj. R^2**
IRR	1	0.0036	0.65	0.4251	0.81
Geno	4	0.0261	4.66	0.0025	
DAR5	1	0.3416	61.01	<0.0001	
DAR5 × Geno	4	0.0196	3.51	0.0125	
DAR5 × DAR5	1	0.5663	101.14	<0.0001	
DAR5 × DAR5 × Geno	4	0.0213	3.80	0.0083	
$y = ax^2 + bx + c$					
Geno	**Relative MG**	**Irri**	**a**	**b**	**c**
S25-E5	2.5	DR/IRR	−0.0003	0.0126	0.5985
S35-A5	3.5	DR/IRR	−0.0006	0.0225	0.5660
R2 36X82N	3.6	DR/IRR	−0.0008	0.0303	0.5407
P46T21R	4.6	DR/IRR	−0.0008	0.0379	0.4328
AG5532	5.5	DR/IRR	−0.0004	0.0066	0.8693

† DF, degrees of freedom. MG, maturity group.

DISCUSSION

The results in the present study indicate that aerial DGCI measurements can serve as a relative metric for soybean canopy N concentration. Additionally, the decrease in aerial DGCI values during seedfill may have applications in determining soybean maturity remotely and in distinguishing differences in response to drought relative to water replete conditions. These results of aerial DGCI are comparable to previous studies with ground DGCI measurements. Rorie et al. (2011) reported a close association between DGCI and leaf N concentration in corn that reached a plateau at leaf N concentration greater than 2%. Hoyos-Villegas et al. (2014) tested the response of DGCI in soybean to drought by using rooting barriers placed at different depths, and found that DGCI values declined with a rooting barrier at 0.3 m compared with the control treatment (i.e., no rooting barrier). Hastened senescence with increased drought conditions (due to shallow rooting) are similar to the results from aerial DGCI in this study. Similarly, Yu et al. (2016) were able to predict soybean maturity with greater than 93% accuracy based on the decrease in NDVI as plants began to senesce.

Aerial DGCI decreased with increasing DAR5 (Fig. 4). These responses were similar to the decrease in leaf N concentration versus DAR5 (Fig. 1), which are indicative of N remobilization from leaves to seed causing leaf yellowing. However, compared to G_DGCI (Fig. 3), aerial DGCI had greater sensitivity for detecting the differences between IRR and DR treatments. Therefore, aerial DGCI measurements have advantages over G_DGCI measurements for identifying effects of drought. The reason for the greater sensitivity of aerial DGCI might be that aerial images covered a larger area than ground images so that aerial DGCI may be more representative than G_DGCI. Another reason could be the angle differences when images were taken. Ground images were taken at an oblique angle with the canopy, but the aerial images were taken vertically above the top of the canopy, which may allow a better assessment of leaf senescence through different strata in the canopy.

Ground imaging measurements covered about 1 m^2 of the top portion of the canopy for each plot, whereas aerial images covered a large number of plots each measurement date. Thus, ground imaging was time-consuming. However, aerial image measurements were highly dependent on weather conditions and required training for flying. The widespread use of unmanned aerial systems greatly simplifies collecting aerial images. Because aerial images discriminated the difference of greenness between irrigation treatments, this method has potential for characterizing soybean genotypes that senesce slowly and are less affected by drought stress.

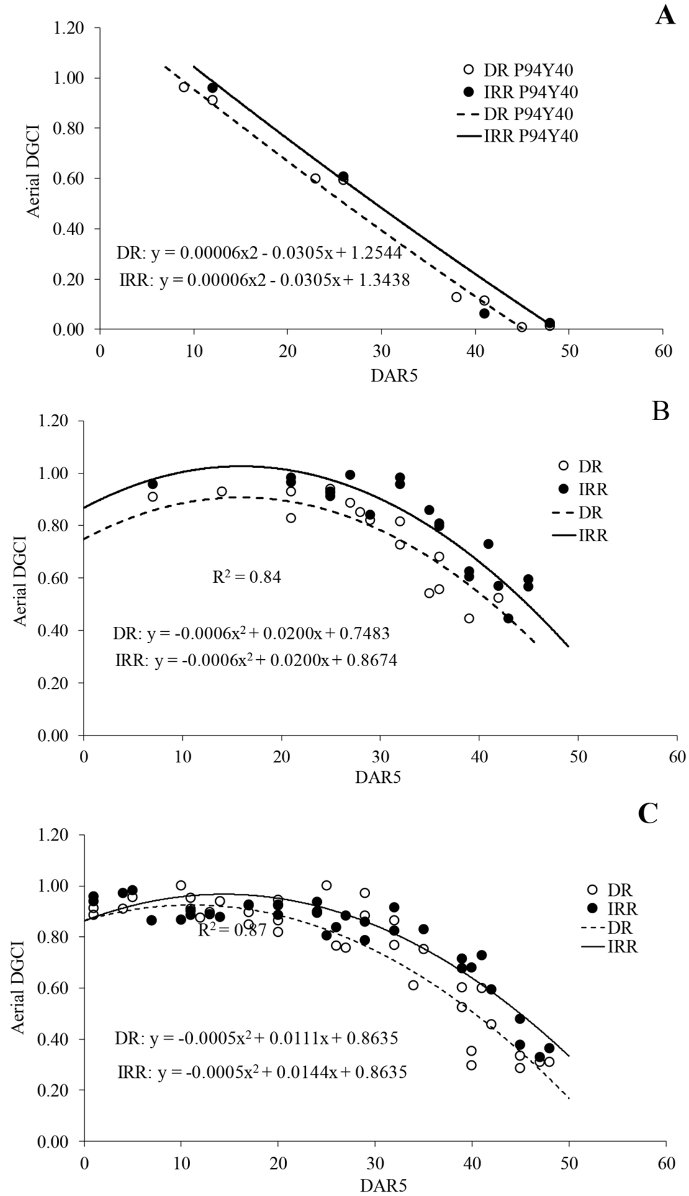

Fig. 4. Aerial dark green color index (DGCI) versus days after Reproductive Stage 5 (DAR5) under drought (DR) and well-irrigated water (IRR) treatments in 2012. Genotype P94Y40 was used to represent the response of aerial DGCI to DAR5, which was similar to other genotypes (not included in the figure; A). Aerial DGCI versus DAR5 across genotypes (not significant) in 2013 (B) and in 2014 (C).

Nitrogen nutrition and N management are at the nexus of crop productivity and environmental stewardship. Further development of aerial DGCI measurements opens the possibility of a relatively simple, high throughput method for a range of issues including: identifying genetic differences in DGCI (as a surrogate measure of canopy nitrogen concentration), remotely determining maturity date for annual species, developing N sufficiency indices for non-legume crops and turf species, and in identifying slow senescence in response to drought in soybean and other crops. The present research lays the foundation for identifying

Table 6. Analysis of covariance (ANCOVA) for aerial dark green color index (DGCI) versus days after Reproductive Stage 5 (DAR5) in 2012, 2013, and 2014. Factors considered include irrigation (Irri), genotype (Geno), DAR5, DAR5 × DAR5, and all two- and three-way interactions. Letters a, b and c represented the quadratic and linear slopes and intercept for each genotype across water treatments in the model. Nonsignificant interactions were removed from the model stepwise.

Aerial DGCI 2012

Source	DF†	Mean Square	F Value	Pr > F	Adj. ^{2}
Irri	1	0.1114	9.60	0.0034	0.89
Geno	4	0.3786	32.61	<0.0001	
DAR5	1	0.3154	27.17	<0.0001	
DAR5 × Geno	4	0.2386	20.55	<0.0001	
DAR5 × DAR5	1	0.8253	71.09	<0.0001	
DAR5 × DAR5 × Geno	4	0.1835	15.81	<0.0001	

$y = ax^2 + bx + c$

Geno	Relative MG	Irri	a	b	c
AG24–30	2.4	DR	−0.0017	0.0623	0.0837
		IRR	−0.0017	0.0623	0.1732
S25-T8	2.5	DR	−0.0021	0.0748	0.0739
		IRR	−0.0021	0.0748	0.1634
S33-K5	3.3	DR	−0.0012	0.0449	0.1781
		IRR	−0.0012	0.0449	0.2676
P94Y40	4.4	DR	0.0001	−0.0305	1.2544
		IRR	0.0001	−0.0305	1.3438
P95Y50	5.5	DR	0.0001	−0.0208	0.8601
		IRR	0.0001	−0.0208	0.9496

Aerial DGCI 2013

Source	DF†	Mean Square	F Value	Pr > F	Adj. ^{2}
Irri	1	0.1109	18.94	0.0002	0.84
Geno	4	0.0040	0.68	0.6135	
DAR5	1	0.0510	8.72	0.0064	
DAR5 × DAR5	1	0.1644	28.08	<0.0001	

$y = ax^2 + bx + c$

Irri	a	b	c
DR	−0.0006	0.0200	0.7483
IRR	−0.0006	0.0200	0.8674

Aerial DGCI 2014

Source	DF†	Mean Square	F Value	Pr > F	Adj. ^{2}
Irri	1	0.0048	0.77	0.3825	0.87
Geno	4	0.0014	0.22	0.9254	
DAR5	1	0.1409	22.53	<0.0001	
DAR5 × Irri	1	0.0382	6.12	0.0162	
DAR5 × DAR5	1	0.5636	90.13	<0.0001	

$y = ax^2 + bx + c$

Irri	a	b	c
DR	−0.0005	0.0111	0.8635
IRR	−0.0005	0.0144	0.8635

† DF, degrees of freedom; MG, maturity group; DR, drought; IRR, well-irrigated.

genetic differences in canopy greenness, and in quantifying slow senesce of genotypes that have similar maturity under water replete conditions.

Conflict of Interest

The authors declare that there is no conflict of interest.

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