ORIGINAL PAPER

# **Seed composition is influenced by irrigation regimes and cultivar**  $\mathbf{r}$ **diVerences in soybean**

**Nacer Bellaloui · Alemu Mengistu** 

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**Abstract** In the midsouth USA, soybean is produced either under irrigated or non-irrigated conditions. The objective of this experiment was to show the utility of supplemental irrigation as an alternative to full-season and non-irrigation to achieve high yield and high seed composition. The effects of irrigation and cultivar differences on soybean yield and seed composition were conducted. Two cultivars (Dwight and Freedom) and three irrigation regimes (full-season irrigation, FS; reproductive stage/supplemental irrigation, RI; and non-irrigation, NI) were used. Protein percentage was higher in Dwight under FS and RI than NI. In Freedom, protein percentage was higher under NI than under FS and RI. Under NI, Freedom had higher protein percentage than Dwight, especially in 2004, but lower oil in 2003 and 2004. Cultivars showed significant differences in fatty acids. Yield in Freedom under FS and RI was not significantly different. Nitrogen fixation was substantially higher under NI conditions. The results indicate that irrigation management and cultivar selection

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N. Bellaloui  $(\boxtimes)$ 

A. Mengistu

significantly affect seed composition and yield. Protein increase in Freedom under non-irrigated conditions may benefit producers for high protein seed under dry-land conditions. Supplemental irrigation at the reproductive stage may be a possible alternative for full season irrigation for the cultivar Freedom.

## **Introduction**

Although the early soybean production systems (ESPS) has been successfully implemented into the southern USA and enhanced productivity under irrigated and non-irrigated conditions (Heatherly et al. [1999\)](#page-6-0), supplemental irrigation at a reproductive stage as an alternative to full-season irrigation and non-irrigation to achieve high yield and high seed composition has not been well investigated. About 50% of soybean grown in the Mississippi delta, mid-south USA is still under non-irrigated production system. This is mainly due to the fact that soybean yield varies from season to season because of environmental conditions of soil type, rainfall, temperature, and management practices (MSUCares [2007\)](#page-6-1). To maintain a higher yield and higher seed quality across years, supplemental irrigation may be needed as a possible alternative to full-season and non-irrigated production system. Soybean seed composition is one of the major components of soybean seed quality. Soybean quality is determined by oil and protein content because soybean seed is a major source of high quality protein and oil (Grieshop and Fahey [2001\)](#page-6-2). Protein in soybean seed ranges from 34.1 to  $56.8\%$ , with a mean of  $42.1\%$ , and oil ranges from 8.3 to 27.9%, with a mean of 19.5% (Wilcox and Shibles [2001\)](#page-7-0). However, protein concentration may vary depending on environmental stresses such as temperature and drought (Howell and Cartter [1958\)](#page-6-3). Severe drought can

Crop Genetics and Production Research Unit, USDA-ARS, 141 Experiment Station Road, Stoneville, MS 38776, USA e-mail: nbellaloui@msa-stoneville.ars.usda.gov; nbellaloui@ars.usda.gov

Crop Genetics and Production Research Unit, USDA-ARS, 605 Airway Boulevard, Jackson, TN 38301, USA

lead to a decrease in protein concentrations (Specht el al. [2001](#page-7-1)). Other researchers reported that severe drought increased protein content by 4.4%, while oil content decreased by 2.9% (Dornbos and Mullen [1992](#page-6-4)).

The concentration of saturated fatty acids ranges from 100 to 120  $g \text{ kg}^{-1}$  of total oil for palmitic acid, and from 22 to  $72 \text{ g kg}^{-1}$  of total oil for stearic acid (Cherry et al. [1985](#page-6-5)). The mean concentration of unsaturated fatty acids is 240 g kg<sup>-1</sup> of total oil for oleic acid, 540 g kg<sup>-1</sup> of total oil for linoleic acid, and 80 g  $kg^{-1}$  of total oil for linolenic acid (Schnebly and Fehr [1993](#page-7-2)). Because of oxidation during deep pot frying, the concentration of polyunsaturated fatty acids, such as linoleic acid, and especially, linolenic acid can be reduced by hydrogenation. Hydrogenation of these unsaturated fatty acid leads to trans-isomers, which are associated with increased incidence of heart disease (Rakow and McGregor [1973](#page-7-3)). However, monounsaturated fatty acids such as oleic acid are less susceptible to oxidative changes during refining, storage, and frying, which are highly desirable. Consequently, the food industry is becoming increasingly interested in producing soybean seed with a high content of oleic acid and low linoleic and linolenic acids (Rahman et al. [2001\)](#page-7-4). Palmitic acid content of various soybean genotypes has been reported with a range of <40 to about 380 g  $kg^{-1}$  (Fehr et al. [1991\)](#page-6-6). Soybean with less than 40 g  $kg^{-1}$  palmitic acid is more desirable for human consumption than the conventional soybean oil, with about 110 g  $kg^{-1}$ . Decreasing palmitic acid content in soybean oil is a desirable trait for human consumption and seed oil industry because of its oxidative stability.

Nitrogen fixation is restricted by environmental factors (Schubert [1995\)](#page-7-5) such as water stress (Guerin et al. [1990](#page-6-7); Pefia-Cabriales and Castellanos [1993](#page-6-8); Sinclair et al. [2007](#page-7-6)). Nitrogen fixation may be limited by environmental factor stresses through *Rhizobium* (*Bradyrhizobium*) multiplication in soil, rhizobial infection of roots, nodulation, and  $N_2$ fixation (Shubert [1995\)](#page-7-5). Although,  $N<sub>2</sub>$  fixation is vulnerable to drought (Sinclair and Serraj [1995](#page-7-7); Serraj et al. [1999\)](#page-7-8), the interaction between the source of nitrogen fixation (atmospheric N) and mineral nitrogen (soil N) is important and need to be understood to maximize legume  $N_2$  fixation and yield (George and Singleton [1992\)](#page-6-9). Frechilla et al. ([2000\)](#page-6-10) reported that growth declined under water deficit, but nodulated plants were less sensitive to drought than nitrate-fed plants. It was concluded that the nitrogen source is a major factor affecting responses to water stress (George and Singleton [1992\)](#page-6-9).

The specific objective of this study was to investigate the effect of irrigation regimes and soybean cultivars on seed composition (oil, fatty acids, and protein concentration), nitrogen fixation using natural  $15N$  abundance method, and yield in ESPS.

#### **Materials and methods**

Field experiments were conducted at Delta Research and Extension Center, Stoneville, MS (33°26' 90°54' LN; LW; 39 m above the sea level), on selected soybean [*Glycine max* (L.) Merr.] cultivars Dwight of maturity group II and Freedom of maturity group V in 2002, 2003, and 2004 for yield and in 2003 and 2004 for seed composition. Stoneville's climate is described as humid subtropical, with temperate winters; long, hot summers. Mississippi's latitudinal location (30°–35° North) imposes an additional seasonal characteristic which influences summer precipitation, leading to period of drought and climatic variability in the growing season (Pote and Wax [1986](#page-7-9); MSUCares [2007](#page-6-1)). The plants were grown under three irrigation regimes (fullseason irrigation, FS; reproductive stage irrigation, RI, and non-irrigation, NI) each year. Each irrigation treatment had eight replicates.

Seeds were treated with mefenoxam  $[(R)-2-(2.6-(dim$ ethylphenyl)-methoxyacetylamino}-propionic acid methyl ester] fungicide prior to planting as a precaution against stand loss due to *Pythium* spp. Row spacing was 0.5 m and seeding rate was  $16-20$  seed  $m^{-1}$  of row. Plots were 23 m long in 2002 and 2003, whereas irrigated plot length was 31 m, and non-irrigated plot length was 21 m in 2004. Plots in all years were 4 m (eight rows) wide.

Soybeans were grown on Sharkey clay (very-fine, smectitic, thermic chromic Epiaquert) and experiments were conducted according to Heatherly ([1999](#page-6-11)), Hearherly et al. ([2001](#page-6-12)) and Ray et al. [\(2006\)](#page-7-10). The three planting were 17 April 2002, 14 April 2003, and 19 April 2004. The harvesting date for Dwight was 11 August 2002, 12 August 2003, and 8 August 2004. Harvesting date for Freedom was 29 September 2002, 2 October 2003, and 26 September 2004. Plots were furrowirrigated according to Heatherly ([1999](#page-6-11)), Hearherly et al. [\(2001](#page-6-12)), and Ray et al. [\(2006\)](#page-7-10). Initial irrigation was applied to FS on 23 May 2002, 31 May 2003, and 9 June 2004. Initial irrigation was applied to RI on 3 June 2002, 26 June 2003 and 18 June 2004. Irrigation was not applied to NI treatment throughout the season. Irrigation was applied thereafter to FS and RI each year whenever soil water potential at the 30 cm depth, as measured by tensiometers, decreased to about  $-50$  kPa (Heatherly [1999](#page-6-11); Heatherly et al. [2001;](#page-6-12) Ray et al. [2006\)](#page-7-10). Irrigation of FS and RI was continued until full seed stage of cultivars. Tensiometers and weather station data have been used and resulted in practical approach to scheduling irrigation for soybeans in research at Stoneville, MS (Heatherly [1999\)](#page-6-11). After irrigation, the range of soil water potential for FS was maintained between 0 and  $-21$  kPa over the growing season till maturity; for RI, the soil water potential ranged between 0 and  $-40$  kPa; for NI, the range of soil water potential was very low, especially during the critical stages of the crop development in June to August and ranged

between  $-100$  and  $-198$  kPa. There were clear differences between the soil water potential values for NI versus the soil water potential values for FS and RI, creating three irrigation environments.

#### Seed analysis for oil, fatty acids, and protein

Approximately 25 g of whole grain from each replicate were analyzed for oil, fatty acids, and protein, using near infrared (NIR) reflectance at the National Center for Agricultural Utilization Research at Peoria, IL, USA (Wilcox and Shibles [2001\)](#page-7-0). The analysis was performed based on a percent (%) dry matter (Wilcox and Shibles [2001](#page-7-0); Boydak et al. [2002](#page-6-13)). Fatty acid analysis was conducted on 2004 samples only.

## Nitrogen fixation using natural  $15N$  abundance method

Delta  $15N$  abundance was evaluated from nitrogen isotope  $15N/14N$  ratio (Delwiche and Steyn [1970](#page-6-14); Shearer and Kohl [1986](#page-7-11); Peoples and Herridge [1990](#page-6-15)) in about 0.9 mg of ground seeds. A Thermo Finnigan Delta Plus Advantage Mass Spectrometer with a Finnigan ConFlo III and Isomass Elemetal Analyzer was used for isotopic analysis. Delta values were obtained using Isodat software version 2.38. The elemental combustion system was Costech ECS 4010 with an autosampler. Nitrogen fixation was measured in 2004 only.

#### Statistical analysis

A split plot design was used with irrigation as a main plot and cultivar as a sub-plot. Eight replicates were used. Results were analyzed by SAS, using Proc GLM. To investigate the source of variability, the main effects of cultivar, year, and their interactions on seed composition were also analyzed using SAS.

## **Results**

Protein percentage was higher in Dwight under FS and RI than NI in 2003 and 2004 (Table [1\)](#page-2-0). In Freedom, however, protein percentage was higher under NI than under FS or RI in 2003 and 2004. Protein and oil percentages in Dwight under FS and RI were not consistent across years, but in Freedom, oil was higher under FS and RI in 2003 and 2004. Cultivar differences affected seed composition only in 2003. where protein in Dwight was highly  $(P < 0.001)$  significant than Freedom under both FS and RI. Oil percentage was also greater in Dwight than in Freedom under FS, RI, and NI in 2004 only (Table [2\)](#page-2-1). Under NI, protein percentage in Freedom was much higher than in Dwight in 2004, and the

<span id="page-2-0"></span>**Table 1** Protein and oil percentages  $(\%)$  as influenced by irrigation regimes for each cultivar in 2003 and 2004

Cultivar	Treatment	2003		2004		
		Protein	Oil	Protein	Oil	
Dwight	FS	44.1a	20.6a	41.7a	21.1 <sub>b</sub>	
	RI	42.3 <sub>b</sub>	20.9a	41.9a	21.4b	
	NI	41.9b	21.2a	37.3 <sub>b</sub>	23.5a	
Freedom	FS	40.7 <sub>b</sub>	21.1a	41.4b	20.1a	
	RI	41.0 <sub>b</sub>	20.7a	41.4b	19.9a	
	NI	42.2a	20.1 <sub>b</sub>	45.5a	17.8b	

Means within a column for each cultivar separately followed by the same letter (s) are not significantly different at  $P \le 0.05$  for each irrigation treatment. Values are means of eight replicates

*FS* full-season irrigation, *RI* reproductive irrigation, *NI* non-irrigation

<span id="page-2-1"></span>Table 2 Protein and oil percentages (%) as influenced by cultivar differences under different irrigation regimes in 2003, and 2004

Irrigation	Cultivar	2003		2004		
		Protein	Oil	Protein	Oil	
FS	Dwight	44.1a	20.6a	41.7a	21.1a	
	Freedom	40.7 <sub>b</sub>	21.1a	41.4a	20.1 <sub>b</sub>	
RI	Dwight	42.3a	20.9a	41.9a	21.4a	
	Freedom	41.0 <sub>b</sub>	20.7a	41.4a	19.9 <sub>b</sub>	
NI	Dwight	41.9a	21.2a	37.3 <sub>b</sub>	23.5a	
	Freedom	42.2a	20.1 <sub>b</sub>	45.5a	17.8b	

Means within a column for each irrigation treatment separately followed by the same letter (s) are not significantly different at  $P \leq 0.05$ for the two cultivars. Values are means of eight replicates

*FS* full-season irrigation, *RI* reproductive irrigation, *NI* non-irrigation

increase of protein was 22% (Table [2](#page-2-1)). This trend was the opposite to what was recorded under FS and RI in 2003, but not in 2004. Oil percentage in Dwight was significantly higher than in Freedom in 2004. For example, in 2004 and under NI, oil increase in Dwight was 32% higher than in Freedom (Table [2\)](#page-2-1).

Analysis of variance showed that year, cultivar, and year  $\times$  cultivar interactions were significant ( $P < 0.05$ ) for both protein and oil. Since year  $\times$  cultivar was significant  $(P < 0.0001$  for protein and oil under FS and NI; *P* < 0.0001 for protein and *P* < 0.02 for oil under RI), data were presented in each year.

## Fatty acids and nitrogen fixation

Since seed of Freedom in 2004 showed higher percentage of protein and lower percentage of oil compared with Dwight, it was decided to investigate further the levels and changes in fatty acids and nitrogen fixation under all irrigation regimes in both cultivars.

<span id="page-3-0"></span>**Table 3** Fatty acid percentages  $(\%)$  as influenced by irrigation regimes in each cultivar in year 2004

	Cultivar Treatment Palmitic Stearic Oleic Linoleic Linolenic					
Dwight	FS	12.8a	3.9 <sub>b</sub>		23.8ab 53.5ab	6.1a
	RI	12.5ab	3.7 <sub>b</sub>	22.6b $54.9a$		6.3a
	NI	12.3 <sub>b</sub>	4.1a	$25.3a - 52.3b$		5.9a
Freedom FS		14.1a	3.9a	$18.0b$ 56.9b		7.2a
	RI	13.4 <sub>b</sub>	3.6 <sub>b</sub>	18.4ab 57.6a		7.0a
	NI	13.6 <sub>b</sub>	4.0a	19.4a 55.9b		7.1a

Means within a column for each cultivar separately followed by the same letter (s) are not significantly different at  $P \le 0.05$  for each irrigation treatment. Values are means of eight replicates

*FS* full-season irrigation, *RI* reproductive irrigation, *NI* non-irrigation

<span id="page-3-1"></span>**Table 4** Fatty acid percentage  $(\%)$  as influenced by cultivar differences under full-season irrigation, FS; reproductive stage irrigation, RI; and non-irrigation, NI

Fatty acids	FS		RI		NI		
		Dwight Freedom Dwight Freedom Dwight Freedom					
Palmitic	12.8 <sub>b</sub>	14.1a	12.5 <sub>b</sub>	13.4a	12.3 <sub>b</sub>	13.6a	
Stearic	3.9a	3.9a	3.7a	3.6a	4.1a	4.0a	
Oleic	23.8a	18.0 <sub>h</sub>	22.6a	18.4h	25.3a	19.4 <sub>b</sub>	
Linoleic	53.5b	56.9a	54.9b	57.6a	52.3b	55.9a	
Linolenic	6.1 <sub>b</sub>	7.2a	6.3 <sub>b</sub>	7.0a	5.9b	7.1a	

Means within a row at each irrigation treatment followed by the same letter (s) are not significantly different at  $P \le 0.05$  for cultivars. Values are means of eight replicates

There was no consistency on the effect of irrigation on fatty acids (Table [3](#page-3-0)). However, our 1-year preliminary results indicated that there was a significant cultivar effect in fatty acids (Table [4\)](#page-3-1). For example, oleic fatty acid was higher in Dwight than in Freedom, but palmitic, linoleic, and linolenic fatty acid percentages were higher in Freedom than in Dwight. Stearic was relatively stable. Nitrogen fixation, as shown by a low  $15N/14N$  ratio, was substantially higher under NI conditions (Fig. [1](#page-3-2)) than under FS or RI.

### Yield

Statistical analysis indicated that irrigation, cultivar, and year were highly significant ( $P \leq 0.01$ ) for yield. Since there was a significant interaction between cultivar and year, yield data were presented in each year. Under FS and RI, yield in Dwight was significantly higher than under NI in 2002 and 2004. In Freedom, yield was significantly higher under FS and RI than under NI in 2002, 2003, and in  $2004$  (Table [5](#page-3-3)). Cultivar showed significant differences in yield. Yield in Freedom was consistently higher than in Dwight in all years, except under NI in 2004 (Table [5\)](#page-3-3).



<span id="page-3-2"></span>**Fig. 1** The effect of irrigation (*FS* full-season, *RI* reproductive/supplemental irrigation, and *NI* non-irrigation) on nitrogen fixation using nitrogen isotope abundance  $({}^{15}N/{}^{14}N$  ratio) in Dwight and Freedom cultivars

<span id="page-3-3"></span>**Table 5** Yield ( $kg \text{ ha}^{-1}$ ) as influenced by irrigation regimes for each cultivar in 2002, 2003, and 2004

Cultivar	Treatment	2002	2003	2004	
			Yield $(kg ha^{-1})$		
Dwight	FS	3,073a	1,767a	1,780a	
	RI	2,731b	1,331b	1,970b	
	NI	1,578c	1,358b	963c	
Freedom	FS	4,943a	4,947a	2,776a	
	RI	4,977a	4.994a	2,650a	
	NI	4,161b	3,338b	904b	

Means within a column for each cultivar separately followed by the same letter (s) are not significantly different at  $P \le 0.05$  for each irrigation treatment. Values are means of eight replicates

*FS* full-season irrigation, *RI* reproductive irrigation, *NI* non-irrigation

## **Discussion**

It was shown that protein percentage was higher in Dwight under FS and RI than under NI in 2003 and 2004. This indicates that Dwight accumulated more protein under irrigated conditions than under non-irrigated conditions. In Freedom, however, protein percentage was higher under NI than under FS or RI in 2003 and 2004. This indicates that cultivars respond differently to irrigation. This response may underline maturity or/and genotype differences and their adaptations to environmental stress factors such as soil water content and temperature. Cultivar differences showed a significant influence as well, suggesting that seed composition qualities depend on the cultivar and type of irrigation used for production. For example, in 2004 and under NI, protein percentage in Freedom was higher than in Dwight by 22%. However, oil was higher in Dwight than Freedom by 32%. The differences in seed composition between the cultivars could be either attributed to cultivar differences (Maestri et al. [1998](#page-6-16); Piper and Boote [1999](#page-6-17)), maturity group differences (Zhang et al.  $2005$ ), or cultivar adaptability to irrigation management. Our results showed that Dwight in 2004 accumulated higher oil (23.5%) and lower protein (37.3%) under NI compared with the accumulated protein and oil under FS and RI. However, Freedom accumulated higher protein (45.5%) and lower oil (17.8%) under NI compared with the accumulated protein and oil under FS or RI conditions. This suggests that increase or decrease in oil may depend on the level of water stress (drought) (Specht et al. [2001\)](#page-7-1), genotype (Maestri et al. [1998](#page-6-16); Piper and Boote [1999](#page-6-17)), and timing of full maturity of these cultivars (Zhang et al. [2005\)](#page-7-12). It was also reported that the interrelationships among seed quality attributes in soybean and lines  $\times$  environments interactions were significant for seed yield, protein, and oil (Wilcox and Shibles [2001\)](#page-7-0). The weather data on rainfall and air temperature (Fig. [2\)](#page-4-0) (MSUCares [2007\)](#page-6-1) showed that the period from June to August is important as it coincides with initial bloom to full bloom (R1–R2) in June; beginning pod to full pod to beginning seed (R3–R4–R5) in July; and full seed to beginning maturity to full maturity (R6–R7–R8) in August. Table [6](#page-5-0) show that during July–August, where the critical stage of seed development (seed fill), water deficit reached  $-128.3$ ,  $-136.4$ , and  $-131.3$  mm, respectively, in 2002, 2003, and 2004. NI treatment must have grown under drought conditions, and this was reflected by soil water potential values that reached upto  $-100$  to  $-198$  kPa in June–August. Our current irrigation research (detailed data not shown), using water potential sensors, indicated that about  $-15$  kPa represent the water field capacity for the soil type described above;  $-50$  to  $-60$  kPa represent a level where water stress occurs and irrigation needs to be applied for higher yield;  $<-100$  kPa represent drought effect that would have a significant impact on yield and seed composition. Our recent data (data not shown) indicated that after a regular irrigation (once per 7–10 days), soybean need about 56.8 mm of water per week to 10 days to avoid water stress. This amount can increase to 76.2 mm, depending on the stage of the crop, size of soil cracks, and irrigation water pressure. Heatherly [\(1999](#page-6-11)) showed that pan evaporation in the region typically is 6.35 mm day<sup>-1</sup> during R1 (beginning of flowering) to R6 (full seed fill). Thus, in the absence of rain, about 76.2 mm of water is needed about every 12 days. This is the normal amount of water applied for furrow irrigation to cracking clay soils (Heatherly [1999](#page-6-11)).



<span id="page-4-0"></span>**Fig. 2** Rainfall (mm) and air temperature (°C) in 2002 (**a**), 2003 (**b**), and 2004(**c**)

Table [6](#page-5-0) (MSUCares [2007](#page-6-1)) showed that there was more negative moisture as the season progress, creating three different irrigation environments across the growing season. As clearly shown, moisture deficit occurs due to low rainfall and high pan evaporation, leading to more negative moisture deficit as the season progress from April through August at Stoneville, MS (33°26' LN); longitude 90.915 W. These conditions cause severe drought stress

Months	2002			2003			2004		
	Rainfall	Pan evaporation	Diff.	Rainfall	Pan evaporation	Diff.	Rainfall	Pan evaporation	Diff
April	82.8	160	$-77.2$	96.0	182.6	$-86.6$	104.65	169.16	$-64.52$
May	71.6	200.7	$-129.1$	64.8	196.6	$-131.8$	184.15	181.86	2.29
June	66.8	192.5	$-125.7$	185.4	193.0	$-7.6$	237.24	171.20	66.04
July	83.6	196.9	$-113.3$	62.5	190.5	$-128.0$	78.23	179.07	$-100.84$
August	70.4	198.6	$-128.3$	38.9	175.3	$-136.4$	54.61	185.93	$-131.32$
September	122.9	173.5	$-50.5$	27.4	161.3	$-133.9$	0.76	188.21	$-187.45$

<span id="page-5-0"></span>Table 6 The total monthly rainfall (mm), pan evaporation (mm), and water deficit (diff.) (mm) for growing season months

Data were obtained from MSUCares, the weather station at Stoneville, Mississippi State University, Extension Service ([http://ext.msstate.edu/anr/](http://dx.doi.org/http://ext.msstate.edu/anr/drec/weather.cgi) [drec/weather.cgi](http://dx.doi.org/http://ext.msstate.edu/anr/drec/weather.cgi)). Verified on 22 August 2007

during nearly every growing season (Heatherly [1999\)](#page-6-11). In ESPS system, the critical stages of development, especially seed fill (July–August) occur during the time of greatest drought stress in normal years, based on the long-term weather data (Heatherly [1999](#page-6-11)). Since the rainfall in 2002, 2003, and 2004, in June, July, and August was less uniformly distributed, contribution of rainfall to seed composition difference under NI cannot be excluded. In 2002 and 2003, the maximum and minimum temperature was near normal. However, in 2004 the temperature was cooler than in 2002 and 2003 (Fig. [2](#page-4-0)). Therefore, we cannot completely exclude the partial influence of rainfall and temperature beside the main factors of cultivar differences and irrigation treatments on seed composition. Using Soypheno software (Zhang et al. [2004a,](#page-7-13) [b\)](#page-7-14), the high rainfall that was taken place by the end of June coincided with beginning of seed fill to full seed  $(R5–R6)$  for Dwight and flowering stage to beginning of pod set (R2–R3) for Freedom. The stages from flowering  $(R2)$  to full seed fill  $(R6)$  are critical for yield and seed composition. Therefore, we conclude that although irrigation and cultivar differences were the main factors influencing seed composition, the effects of rainfall and temperature cannot be excluded.

There was no consistency on the effect of irrigation on fatty acids, but our 1-year preliminary results suggested that there was a significant cultivar effect on fatty acids. For example, oleic fatty acid was higher in Dwight than in Freedom, but palmitic, linoleic, and linolenic fatty acid percentages were higher in Freedom than in Dwight. Stearic was relatively stable. Maestri et al. ([1998\)](#page-6-16) found that saturated fatty acids (stearic and palmitic acids) were stable over regions. However, there was a significant influence of environments on unsaturated fatty acids (oleic, linoleic, and linolenic). In addition, they found that there was a significant varietal effect, but no significant genotype  $\times$  environmental factor interactions was noticed. Our results are in agreement with those above (Maestri et al. [1998\)](#page-6-16) in that there was a significant varietal effect on fatty acids, and the saturated fatty acid stearic was relatively stable. Increasing total oil may lead to an increase in desirable (more stable fatty acids) unsaturated fatty acids such as oleic acid. For example, Dwight under NI accumulated 23.5% total oil with 25.3% oleic acid and 5.9% linolenic acid, whereas, Freedom accumulated 17.8% of total oil with 19.4% oleic acid and 7.1% linolenic acid. Since fatty acids were relatively stable under FS, RI, and NI, we suggest that cultivar selection may be more important than irrigation management for fatty acids production, but both cultivar selection and irrigation management are important for total protein and total oil production. A clear conclusion on the effect of irrigation on fatty acids is not possible at this time and further investigation is needed.

Our yield results are in agreement with those of Heatherly et al. ([1999](#page-6-0)). The non-significant difference in yield under FS and RI in 2002, 2003, and 2004 in Freedom is important, suggesting that RI can be an alternative supplemental irrigation for FS or NI in some soybean cultivars  $(Table 5)$  $(Table 5)$ .

#### Nitrogen fixation

Nitrogen fixation as measured by natural  $^{15}N$  isotope abundance method showed that there was a significant  $(P < 0.0001)$  nitrogen isotope abundance of delta <sup>15</sup>N under FS and RI compared to under NI (Fig. [1\)](#page-3-2). The lower  $15N/14N$ ratio observed in Dwight and Freedom under NI than under FS and RI indicates that both cultivars took up more  $14N$ (atmospheric nitrogen) than  ${}^{15}N$  (soil nitrogen), suggesting that nitrogen fixation was substantially higher under NI conditions. The higher uptake of  $14N$  and lower uptake of  $15$ N under NI may suggest that the source of nitrogen used in nitrogen metabolism may depend on the level of sensitivity of nitrogen fixation and nitrogen assimilation to water moisture in soil. Although it was reported that growing soybean under water deficient condition will lead to nitro-gen fixation inhibition (Purcell et al. [2000](#page-7-15); Wilson [2004](#page-7-16)). Frechilla et al. [\(2000](#page-6-10)) reported that growth declined under water deficit, but nodulated plants were less sensitive to

drought than nitrate-fed plants. They concluded that the nitrogen source is a major factor affecting responses to water stress, although the difference in sensitivity seems to be related to complex interactions with photorespiratory flux and stomatal conductance.

Based on our results and the above discussion, it can be suggested that nitrogen fixation is less sensitive than nitrogen assimilation under water stress. This is may be one of the mechanisms used to maintain nitrogen level in seeds under environmental stresses such as under drought where nitrate uptake by roots and root nodules may be inhibited. This observation, when confirmed, would have a significant agriculture application for potential yield increase through maintaining an optimum *Rhizobium* level under non-irrigated/dry-land conditions.

## **Conclusions**

Irrigation management and cultivar differences have a significant influence on yield and seed composition. Dwight appears to have higher protein, oil, and oleic fatty acid accumulation than Freedom under full-season and reproductive irrigation conditions. Freedom, however, appears to have higher protein, linoleic, and linolenic accumulation under non-irrigated conditions. Information on cultivar differences in seed composition under different irrigation regimes can be useful for soybean breeders for seed composition germplasm development. Yield data show that Freedom had significantly higher yield under irrigated condition than non-irrigated conditions. Reproductive irrigation may be a possible alternative for full season irrigation for a higher yield without sacrificing seed composition qualities. The economics of this possibility needs to be investigated to show profitability and net return. Nitrogen fixation using nitrogen isotope showed that Dwight and Freedom used different sources of nitrogen, depending on the irrigation regimes. This observation is interesting and needs further research. If this observation confirmed, it would have a significant agriculture application for potential yield increase through maintaining an optimum level of *Rhizobium* under non-irrigated/dry-land conditions. Since Freedom had a greater protein concentration under non-irrigated conditions than Dwight, this could be of significant agricultural application for protein production for ESPS under dry land conditions. Under irrigated production system, however, producers benefit from a higher yield in Freedom compared to the yield produced under non-irrigated conditions.

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