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RESEARCH

Water Deficit Modulates the Relationship between Temperature and Unsaturated Fatty Acid Profile in Soybean Seed Oil

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ABSTRACT

Soybean [*Glycine max* (L.) Merr.], a major source of vegetable oil worldwide, contains unsaturated fatty acids (UFA) beneficial for human health, leading to the use of soybean oil in nutraceuticals. The objective of this study was to quantify the effect of water deficit on relationships of soybean seed UFA with temperature and with solar radiation. The UFA profile was determined in harvested seeds of commercial cultivars grown in 76 environments in Argentina (29–38° S latitude). To our knowledge, this is the first study demonstrating a differential response of oleic (Ol), linoleic (Li), and linolenic (Ln) acids and Ol/(Li + Ln) ratio to temperature during seed fill (Tm_{R5R7}) under different conditions of field water availability. This study is also the first showing that in environments without water restrictions, Ol, Li, and Ol/(Li + Ln) ratio exhibit a quadratic response to Tm_{R5R7}. In drought environments, rising Tm_{R5R7} and water deficit caused linear increases in Ol and [Ol/(Li + Ln)] ratio and linear decreases in Li and Ln, with both climatic factors exhibiting additive effects on UFA. Cumulative solar radiation predicted UFA in both environments but was less robust than Tm_{R5R7} and precipitation minus potential evapotranspiration during the reproductive period (pp – PET_{R1R7}). Our results demonstrate that water deficit (estimated as pp – PET_{R1R7}) modulates the relationship between Tm_{R5R7} and soybean seed UFA under rainfed conditions.

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Abbreviations: INTA, National Institute of Agricultural Technology; Li, linoleic; Ln, linolenic; Ol, oleic; pp − PET_{R1R7}, precipitation minus potential evapotranspiration during the reproductive period; ϕPSII, quantum yield of Photosystem II; Sr_{R5R7}, cumulative solar radiation during seed fill; Tm_{R5R7}, average daily mean air temperature during seed fill; UFA, unsaturated fatty acids.

Soybean [*Glycine max* (L.) Merr.] is second after palm (*Elaeis guineensis* Jacq.) worldwide in vegetable oil production and consumption (FAS-USDA, 2016). Soybean oil is annually used for foods and food processing applications, such as cooking, salad oil, dressing, mayonnaise, and confectionery coating (Ali, 2010). Soybean oil is also used for nonedible applications, including the production of biodiesel, lubricants, cleaning agents, synthesis of plastics, coatings, adhesives, and manufacture of inks, paints, varnishes, and resins (Cahoon, 2003; Lee et al., 2007). Suitability of soybean oil for a particular end use is determined by its fatty acid composition, which influences its physical and chemical characteristics. Commodity soybean oil is composed of 62% polyunsaturated fatty acids (54% linoleic [Li] and 8% linolenic [Ln]), 23% monounsaturated fatty acid (23% oleic [Ol]), and 15% saturated fatty acids (11% palmitic acid and 4% stearic acid) (Gunstone et al., 2007). Market requirements for soybean oil are opposite. On the one hand, food preparation and oil and biodiesel industry require oil with low oxidation capacity, heat stability, and extended shelf life (rich in Ol). On a smaller scale, oil for direct human consumption should exhibit enhanced nutraceutical value

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high in essential polyunsaturated fatty acids (Li and Ln). As observed in other seed chemical components (i.e., protein, oil, aminoacids, tocopherols, and isoflavones), environmental conditions during seed development also influence fatty acid composition. The effects of temperature on unsaturated fatty acids (UFA) composition of seed oil were assessed and confirmed several years ago (Howell and Collins, 1957; Wolf et al., 1982; Carver et al., 1986). Later studies demonstrated that Ol increases while Li and Ln concentrations decrease with increasing temperature during seed filling (Dornbos and Mullen, 1992; Tsukamoto et al., 1995; Oliva et al., 2006). Moreover, Carrera et al. (2011) concluded that warm environments enhance Ol/Ln ratio (with increases in Ol and decreases in Ln with increasing temperatures), whereas temperate to cool environments promote high levels of essential fatty acids (Li, Ln). However, besides temperature, other factors appear to influence UFA of soybean seed. Water deficit during seed filling has been shown to increase Ol while decreasing Li and Ln concentrations (Dornbos and Mullen, 1992; Bellaloui et al., 2013). Boydak et al. (2002) observed that Ol and Li were significantly higher and lower, respectively, in seed oil when crop irrigation frequency was lowered. Kane et al. (1997) analyzed the relationship between total rainfall during seed fill and fatty acid concentrations and found a significant negative association with Ol and a positive one with Li. Later, Gao et al. (2009) reported a curvilinear model with a quadratic tendency to describe the relationship between precipitation and UFA. In this model, Ol decreased with increasing precipitation up to 302 to 305 mm and then increased, whereas Li and Ln concentrations increased with higher rainfall. Furthermore, intercepted solar radiation during seed filling affected oil fatty acid composition in soybean. For instance, Izquierdo et al. (2009) reported that Ol increased and polyunsaturated fatty acid concentrations decreased under higher intercepted solar radiation.

Although progress has been made towards understanding the effects of temperature and drought during seed fill on accumulation of individual fatty acids in soybean seed oil, knowledge of the combined effect of both climatic variables is limited. An attempt to address this topic was made by Dornbos and Mullen (1992), who could not draw a clear conclusion because of the observed inconsistent effect of drought on fatty acid composition under increasing air temperature in greenhouse experiments. A more recent study conducted under field conditions (Carrera et al., 2015) found that seed oil from heat- and water-stressed plots had higher Ol than seed oil from irrigated plots. On the other hand, Li and Ln decreased under water stress and increased under irrigated conditions. Environmentally, heat and water stress are the most important abiotic factors limiting crop productivity (Mittler, 2006) and alter seed chemical composition (Medic et al., 2014; Carrera and Seguin, 2016), thus compromising food security. To our knowledge, the effects of temperature and water deficit interaction during seed fill on soybean seed oil composition has not been quantified under field conditions. The increasing threat of climatological extremes including high temperatures and drought predicted by the Intergovernmental Panel on Climate Change (IPCC, 2013) could have a dramatic impact on oil quality needed by processors. Therefore, the aim of this research was to study how water deficit modulates the relationship between soybean seed UFA profile, temperature, and solar radiation during seed filling to identify how these climatic variables individually affect the fatty acid components of soybean seed oil.

MATERIALS AND METHODS

Field Data Collection

Soybean commercial cultivars included in the study were (maturity group is given between parentheses): DM 3100 RR (III), DM 3700 RR (III), DM 4400 RR (IV), DM 4600 RR (IV), DM 4800 RR (IV), A 5520 RG (V), A 6445 RG (VI), A 7636 RG (VII), and A 8000 RG (VIII). Seeds of these cultivars were provided by the Soybean Network for Testing Cultivars from the National Institute of Agricultural Technology (INTA), during the 2001–2002 to 2003–2004 crop years. These seeds came from 76 multienvironment yield trials across the Argentine soybean growing region, covering a latitudinal range from 29 to 38° S. The experimental field trials were laid out as a randomized complete block design with three blocks per environment, defined as year, site, and sowing date combinations. The environments used in this study are listed in Table 1. Since the objective of the present study was to explain the response of soybean oil UFA concentrations to climatic variables, rather than considering cultivar effects, the trial dataset was collected to capture the highest environmental variability possible rather than evaluating all cultivars in all environments. Nonetheless, the trial data included one cultivar common to all environments (DM 4800 RR), which was one of the most popular cultivars across the Argentine soybean region. Environments were selected taking into account that the thermal ranges experienced by short (III–V) and long (VI–VIII) maturity groups were similar and as wide as possible. In the selected environments, mean daily temperature during seed filling was similar for both short and long maturity groups (21.8 ± 2.7 and 21.3 ± 2.3°C, respectively). Chemical (nitrate nitrogen, phosphorus, and pH) and physical (percentage of organic matter, bulk density, and texture) analyses of soil samples from each site did not indicate any physical or nutritional constraint on crop development. Crops were grown under rainfed conditions, and cultural practices recommended by INTA's National Soybean Network for testing cultivars were followed, including control of diseases, insects, and weeds to avoid yield reduction and/or effects on seed composition. Plant density in all trials was ~35 to 40 plants m^{-2} at 0.52-m row spacing. Seeds were harvested, and a 300-g grain sample from three replications of each cultivar at each site and from each sowing date was stored in a walk-in refrigerator at 4°C until analyses to avoid enzymatic degradation of oil. Average daily local temperature and solar radiation data were obtained for each crop season and site from INTA

Table 1. Year, site, and sowing dates (day of the month) involved in the 76 multienvironment field trials conducted across the Argentine soybean crop area during 2001 to 2004 crop seasons.

Year and Site	Latitude, longitude	Sept.	Oct.	Nov.	Dec.	Jan.
2001–2002						
Paraná	31°44′ S, 60°32′ W	23	–	12	17	15
Manfredi	31°49′ S, 63°46′ W	–	12	27	–	–
Concepción del Uruguay	32°29′ S, 58°14′ W	–	–	22	7	2, 31
Marcos Juárez	32°41′ S, 62°06′ W	12	11	14	5	–
General Pico	35°40′ S, 63°44′ W	–	5	6	6	3
2002–2003						
Reconquista	29°40′ S, 59°12′ W	26	23	–	12	9
Paraná	31°44′ S, 60°32′ W	23	10	12	26	13
Manfredi	31°49′ S, 63°46′ W	–	–	1, 27	27	–
Concepción del Uruguay	32°29′ S, 58°14′ W	23	23	22	26	–
Marcos Juárez	32°41′ S, 62°06′ W	23	2	5	1	5
Balcarce	37°52′ S, 58°15′ W	–	25	15	5, 23	–
Bellocq	38°20′ S, 60°13′ W	–	9, 30	13	–	–
2003–2004						
Reconquista	29°40′ S, 59°12′ W	30	–	–	11	6
Rafaela	31°10′ S, 61°28′ W	29	–	14	19	–
Córdoba	31°25′ S, 64°11′ W	24	–	4	–	6
Manfredi	31°49′ S, 63°46′ W	–	–	–	1, 29	
Concepción del Uruguay	32°29′ S, 58°14′ W	29	15	14	15, 30	
Marcos Juárez	32°41′ S, 62°06′ W	–	–	21	–	7
General Pico	35°40′ S, 63°44′ W	–	14	–	3	8
Balcarce	37°52′ S, 58°15′ W	–	2	3	–	5
Barrow	38°19′ S, 60°14′ W	–	24	21	29	–
Bellocq	38°20′ S, 60°13′ W	–	14	19	23	–

Meteorological Network Stations, up to 10 km away from each experimental site. Precipitation was recorded from rain gauges located at each experimental plot site. Potential evapotranspiration was calculated using Penman (1948) equation. Phenological stages R1 (beginning flowering), R5 (beginning seed), and R7 (physiological maturity) were recorded in the field in all trials using the scale of Fehr et al. (1971). The combination of daily climatic data and crop stages allowed us to construct a set of climatic variables for each environment. This includes average daily mean air temperature during seed fill (Tm_{R5R7}, °C), cumulative solar radiation during seed fill (Sr_{R5R7}, MJ m^{-2}, calculated as the sum of daily solar radiation during that period), and precipitation minus potential evapotranspiration during the reproductive period (pp − PET_{R1R7}, mm). The latter variable was included because available water during the reproductive period considers moisture in the soil profile, which influences water availability during the seed-filling period (Carrera et al., 2009). The variable pp − PET was used as a simple indicator of water stress. These set of climatic variables summarized the environmental conditions, especially during seed fill, when dry mass accumulation of chemical components occurs (Wilson, 2004).

Analyses of the Unsaturated Fatty Acid Profile

Oil concentration (expressed as percentage of dry matter) was extracted using Twisselmann equipment, as specified by the Official Methods of American Oil Chemists' Society (Ac3-44; AOCS, 1998), with *n*-hexane solvent. The methyl esters of the three major UFA (Ol, Li, and Ln), expressed as percentage in the oil, were prepared from the extracted oil following AOCS Ce 1-62 (AOCS, 1998). After preparation of methyl esters of UFA, samples were analyzed using a 6890 Agilent GC-FID (gas chromatography with flame ionization detector) equipped with a 30-m × 0.32-mm × 0.5-μm capillary column HP-INNOWAX (Crosslinked Polyethylene Glycol) (Agilent Technologies). Results were recorded in a ChemStation Data System. Concentrations of Ol, Li, and Ln methyl esters were corrected with external standard calibration curves. Standard fatty acid mixtures (FAME Mix Rapeseed, AOCS), purchased from Sigma-Aldrich, were used as calibration standards for identification and quantification of peaks. The Ol/(Li + Ln) ratio was constructed because this relationship is considered a general indicator of oil quality (Gao et al., 2009).

Statistical Analyses

Multiple linear regression was performed to model the concentrations of the three major UFA, Ol, Li, and Ln, and the Ol/(Li + Ln) ratio as functions of Tm_{R5R7}, Sr_{R5R7}, and pp − PET_{R1R7}, as well as combinations of climatic variables (Tm_{R5R7} with pp − PET_{R1R7}, Sr_{R5R7} with pp − PET_{R1R7}). A stepwise variable selection procedure was run to identify significant variables at the 5% significance level (used for the *t* test on regression parameter estimates). Model selection was based on adjusted determination coefficient, Mallow's Cp coefficients, and residual analyses. All calculations were made using the statistical software Infostat (Di Rienzo et al., 2015). The Sr_{R5R7} was a significant predictor for Ol, Li, and Ln acid concentrations, but the fitted models were not as good as the models including Tm_{R5R7} and pp − PET_{R1R7} (data not shown). Therefore, we considered the multiple regressions containing Tm_{R5R7} and pp − PET_{R1R7} as predictors. The regressions were performed for each type

of environment, which were divided into two groups on the basis of water availability: non-water-stressed environments (pp − PET_{R1R7} > 70 mm, range 70 to 417 mm) and water-stressed environments (pp − PET_{R1R7} < 70 mm, range −373 to 70 mm). The threshold value of 70 mm for pp − PET_{R1R7} for environment classification was suggested by previous analyses of partial residues of multiple regression, including Tm_{R5R7} and pp − PET_{R1R7} for oil concentration (consisting approximately of 85% of Ol, Li, and Ln acid concentrations) using the same database (Carrera et al., 2009).

RESULTS AND DISCUSSION

Environmental Variation of Unsaturated Fatty Acids Composition

Differences between minimum and maximum values of UFA across the 76 environments (cultivar, year, site, and sowing date combinations) were 178% for Ol, 65% for Li, and 170% for Ln concentrations (Table 2). This difference was 271% for the Ol/(Li + Ln) ratio, the general indicator of oil quality. The variability in oil composition was the result of the different environmental conditions to which soybean seeds were exposed during development. A wide range of variation was also observed for Tm_{R5R7} (14.1–26.6°C), Sr_{R5R7} (220.3–1676.7 MJ m^{-2}), and pp − PET_{R1R7} (−373.0–416.5 mm), as shown in Table 2. Values of UFA and Ol/(Li + Ln) ratio are consistent with those reported by Bologna et al. (2011) and Carrera et al. (2014a) for nontransgenic soybean genotypes tested in the Argentine crop region. The values were also similar to those reported by Schnebly and Fehr (1993), who analyzed two genotypes during 3 yr, and by Gao et al. (2009), who analyzed three genotypes during 5 yr, both in a single location in the United States. By contrast, Kumar et al. (2006) evaluated seven cultivars across four locations in India. They reported higher values for Ol (27.11%) and lower ones for Li (48.87%) and Ln (6.85%) concentrations than values of the aforementioned studies, including our results. Likewise, Tsukamoto et al. (1995) conducted a multisite field trial with seven Japanese cultivars and found higher Ol (29.72%) and lower Li (46.71%) and Ln (6.78%) concentrations. Not surprisingly, the Ol/(Li + Ln) indicator was higher in the latter two studies compared with values of the previously mentioned studies, including our results. The differences observed between reports by Kumar et al. (2006) and Tsukamoto et al. (1995) and the values obtained in our work is likely due to the environmental conditions during soybean seed development. In the previous two studies, mean temperatures during seed fill were higher (22.9 and 23.0°C, respectively) than the average 21.6°C (Table 2) recorded in our multienvironment trial.

Unsaturated Fatty Acids Composition in Non-Water-Stressed Environments

Soybean oil contains more than 99% triglycerides; each triglyceride consists of one glycerol that links three fatty acids, of which Ol, Li, and Ln make up ~85% (Liu, 1997). Based on oil behavior observed in our previous work using the same database (Carrera et al., 2009), we performed multiple linear regressions for the three major UFA: Ol, Li, and Ln, and the Ol/(Li + Ln) ratio for each type of environment (non-stressed and water-stressed environments). In non-water-stressed environments (pp − PET_{R1R7} > 70 mm, range 70 to 417 mm), a quadratic model explained the response of Ol to Tm_{R5R7}, which indicated that Ol concentration decreased with increasing temperature up to 19.0°C and then increased (Fig. 1A) at higher temperatures. The opposite trend was observed for Li, which increased with increasing temperature up to 19.0°C and then decreased (Fig. 1B) at higher temperatures. For Ln, the regression indicated that this fatty acid responded linearly and negatively to Tm_{R5R7}, with a slope of −0.52% $°C^{-1}$ (Fig. 1C). The patterns of Ol and Li (accounting for 77% of the total fatty acids) was reflected in the Ol/(Li + Ln) ratio, which also exhibited a quadratic response to temperature (Fig. 1D). In environments without water stress, mean daily temperature during seed filling explained 52, 41, 81, and 51% variation in total Ol, Li, and Ln concentrations, and Ol/(Li + Ln) ratio; these results show that temperature is the major factor affecting soybean seed UFA concentration. Accordingly, Howell and Collins (1957) found daytime greenhouse temperature as the main environmental factor affecting polyunsaturated fatty acids, compared with other factors such as photoperiod, light intensity, and soil nutrient content. Moreover, a multienvironment field study conducted in Argentina indicated that average mean daily temperature from R5

Table 2. Average, standard deviation (SD), and ranges for unsaturated fatty acid concentrations and oleic to linoleic plus linolenic acid ratio [Ol/(Li + Ln)] for soybean seed samples, average daily mean air temperature during seed fill (Tm_{R5R7}), cumulative solar radiation during seed fill (Sr_{R5R7}), and precipitation minus potential evapotranspiration during the reproductive period (pp – PET_{R1R7}) of the 76 environments from the Argentine crop region.

Variable		Average	SD	Minimum	Maximum
Chemical	Oleic	22.03	4.05	14.7	40.85
	Linoleic	52.17	3.20	35.49	57.44
	Linolenic	7.95	1.49	5.20	14.03
	Ol/(Li + Ln)	0.38	0.10	0.21	0.78
Climatic	Tm_{R5R7} (°C)	21.57	2.54	14.10	26.60
	Sr_{R5R7} (MJ m^{-2})	693.49	278.81	220.25	1676.70
	pp – PET_{R1R7} (mm)	17.87	166.27	–373.00	416.50

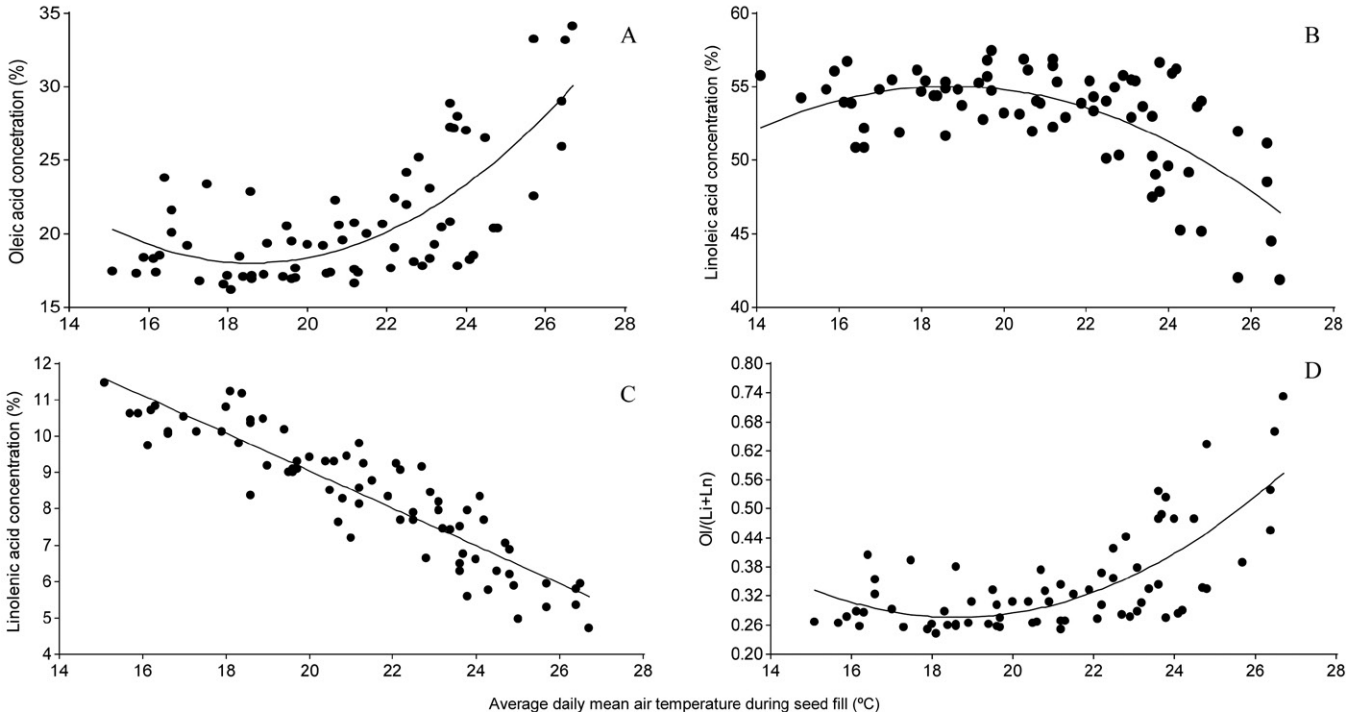

Fig. 1. Response of concentration of (A) oleic acid, (B) linoleic acid, (C) linolenic acid, and (D) oleic to linoleic plus linolenic acid ratio [Ol/(Li+Ln)] in soybean oil to average daily mean air temperature during seed fill (°C). The line represents a fitted linear model for samples from non-water-stressed (precipitation – potential evapotranspiration during the reproductive period > 70 mm) environments (year, site, and sowing date combinations). The following models were computed: (A) $Y = 80.67 - 6.75X + 0.18X^2$, adjusted $R^2 = 0.52$, $P_X < 0.0001$, $P_{X2} < 0.0001$; (B) $Y = 9.02 + 4.94X - 0.13X^2$, adjusted $R^2 = 0.40$, $P_X = 0.0002$, $P_{X2} < 0.0001$; (C) $Y = 19.43 - 0.52X$, adjusted $R^2 = 0.81$, $P < 0.0001$; (D) $Y = 1.67 - 0.16X + 0.004X^2$, adjusted $R^2 = 0.53$, $P_X = 0.0001$, $P_{X2} < 0.0001$.

to R7 was the best predictor of UFA concentrations, compared with mean solar radiation and precipitation minus potential evapotranspiration (Carrera et al., 2011). However, these results disagree with those observed by Gao et al. (2009), who conducted an experiment during 5 yr in one location and failed to establish a relationship between temperature (maximum, mean, or minimum) and oil fatty acid profile, possibly because the average daily temperature during the 5 yr of study was fairly constant.

Sensitivity of UFA composition in response to alterations in environmental temperature is well documented. There is typically an inverse relationship between oil seed polyunsaturated fatty acids (Li and Ln) and temperature during seed development of several oilseed crops, including soybean (Slack and Roughan, 1978; Dwivedi et al., 1996; Rondanini et al., 2003). Several studies have indicated that with increasing temperature during seed development, Ol concentrations increase and Li and Ln concentrations decrease in soybean seed oil. However, to our knowledge, the quadratic response of Ol and Li to temperature as shown in our study has not been previously reported. Within the 14.1 to 19.0°C range, our results indicate a decrease in Ol concentrations and an increase in Li concentrations, resulting in a decrease of Ol/(Li + Ln) ratio up to 18.1°C. However, the opposite effect is shown as temperatures increased above 19°C (Fig. 1D). These results are in contrast with findings of previous research, including our earlier study evaluating nontransgenic genotypes in a similar agroecological area but involving a narrower range of temperatures than in this study (Carrera et al., 2011). One possible explanation of the observed decrease in Ol and increase in Li at temperatures below 19°C could be that at low temperatures, the desaturation from Ol to Li increases as the activity of the desaturation enzymes increases with rising temperature. Early research proposed that this could be in part attributed to increases in oxygen concentration in solution, which is known to be a rate-limiting cofactor in nonphotosynthetic tissue (such as seeds), required for activities of desaturases (Harris and James, 1969). Several studies attempted to explain the relative increase in polyunsaturated fatty acids at low growth temperatures via genetic approaches according to the regulation of the UFA biosynthetic pathways using isolated low (18:3) and/or high (18:1) soybean mutants (Cheesbrough, 1989; Rennie and Tanner, 1989; Kinney, 1994; Heppard et al., 1996). Moreover, Rennie and Tanner (1989) evaluated the effect of contrasting temperatures on soybean mutant C1640 with low Ln concentration (3.4%) and found that at day/night temperatures of 15/12°C, the levels of Ln were much higher than those observed at 28/22°C, and that the high levels of Ln were accompanied by high Li levels and were offset by low Ol levels. Given these results, Kinney (1994) suggested that cold temperatures

induced the expression of genes encoding the enzyme ω-3 desaturase, which catalyzes the conversion of Li to Ln. Likewise, in *Arabidopsis*, the gene encoding ω-3 fatty acid desaturase appears to be induced by cold temperature (Gibson et al., 1994). Genes regulating and encoding the enzyme ω-6 desaturase (the enzyme that catalyzes the conversion of Ol to Li) are poorly understood. Heppard et al. (1996) demonstrated that the increase in Li at low temperatures is apparently not due to transcriptionally induced or enhanced expression of ω-6 desaturase genes in soybean plants; they concluded that this increase is more likely the result of translational and posttranslational regulation, such as altered desaturase activity, as shown by Cheesbrough (1989). Tang et al. (2005) provided evidence for two such posttranscriptional mechanisms. Subsequent research (Byfield and Upchurch, 2007) suggested that the temperature-dependent alteration of Ol and Li in seed-storage lipids may be mediated by transcriptional as well as posttranscriptional mechanisms.

Temperatures rising from 19.0 to 26.7°C caused an increase in Ol of 66.6% and a decrease in Li of 18.1%, resulting in a 119.4% increase in Ol/(Li + Ln) ratio (Fig. 1A, 1B, and 1D). Within the range of temperatures (from 14.1 to 26.7°C) in this study, Ln decreased linearly by 54.2% with rising temperature (Fig. 1C). Wilcox and Cavins (1992) observed a similar percentage decrease of Ln concentration (49.8%) in soybeans exposed to a field range of temperatures varying from 19 to 27°C during seed development. In previous multienvironment field experiments, increased temperature from 22.5 to 24.4°C coincided with a 14.02% increase in Ol and a corresponding decrease in Li of 6.04% and in Ln of 11.27% (Carver et al., 1986). Tsukamoto et al. (1995) evaluated seven Japanese genotypes grown in one location in 1 yr and observed a 76% increase of Ol and a 25 and 35% decrease of Li and Ln, respectively when mean day temperature increased by 1.76°C from 25.4°C during seed development. These results showed that of the three UFA, Ol was the most responsive to temperature during seed development, with increment increases in Ol being higher than the corresponding decreases of Li and Ln. Clearly, the magnitude of the response depends on the temperature increments and is exacerbated under control experiments where soybean is exposed during the whole seed filling to higher temperatures than those that can be reasonably expected to occur in most soybean fields during this period. For instance, soybean plants exposed to day/night phytotron temperature of 33/28°C, resulted in an increase in Ol of 195.4% and reductions of Li and Ln of 27.8 and 69.5%, respectively, compared with plants grown at 18/13°C during seed development (Wolf et al., 1982). Rennie and Tanner (1989) observed that Ol increased by 153.0%, whereas Li and Ln decreased by 48.2 and 60.7%, respectively, in plants exposed to extremely high day/night temperature (40/30°C) during seed development compared with the control (exposed to 28/22°C). The higher proportional increase of Ol concentration than the lower proportional decrease in Li and Ln concentrations with increasing temperature suggests differential sensitivity of the key enzymes ω-6 and ω-3 desaturases to increasing temperatures. Accordingly, Martin et al. (1986) observed that the conversion of Ol to Li was more sensitive to temperature than the conversion of Li to Ln in a soybean genotype (N78-2245) carrying a natural recessive mutation in the gene that encodes the ω-6 desaturase. In seeds of this genotype, the sensitivity of ω-6 desaturase activity to changes in growth temperature was such that the relative ω-6 desaturation decreased 45% when temperature increased by 12°C from 15.5°C, resulting in Ol increases of 81% (Almonor et al., 1998). Noticeably, ω-3 desaturase activity in seeds of this genotype was relatively constant within the same range of growth temperatures (15.5–27.5°C). Although negligible activity of both ω-6 and ω-3 desaturases at 35°C has been reported (Cheesbrough, 1989), degradation of ω-6 desaturase occurred before reaching that temperature, at 30°C (Tang et al., 2005). Further research is needed to quantify the relative sensitivity of these key enzymes to high temperatures, as well as to clarify the mechanisms by which soybean seed fatty acid composition is adjusted to ensure a feasible seed development program under various temperature regimes.

Unsaturated Fatty Acids Composition in Water-Stressed Environments

In water-stressed environments (pp − PET_{R1R7} < 70 mm, range −373 to 70 mm), regression equations for Ol, Li, and Ln concentrations, as well as Ol/(Li + Ln) ratio, showed that not only Tm_{R5R7} but also pp − PET_{R1R7} had a significant effect on UFA concentrations ($p < 0.0001$, Table 3). The adjusted coefficients of determination (adjusted R^2) of these regressions were 0.38, 0.29, 0.70, and 0.39 for Ol, Li, Ln, and Ol/(Li+Ln) ratio, respectively. In addition, Mallow's Cp indicated that the relative contribution of pp − PET_{R1R7} in the fitted regression models increased from approximately a half to a similar magnitude of Tm_{R5R7}. In the case of Li, pp − PET_{R1R7} doubled the Tm_{R5R7} magnitude (Table 3). Interestingly, in these environments, the regression coefficients linearly relating Tm_{R5R7} to Ol, Li, and Ol/(Li + Ln) ratio were 0.57, −0.29, and 0.013% $°C^{-1}$ (Table 3), respectively, being significantly lower than the analogous coefficients in absolute values obtained from the analysis of samples from non-water-stressed environments [−6.86, 5.02, and −0.17% $°C^{-1}$ for Ol, Li, and Ol/(Li + Ln) ratio, respectively, Fig. 1]. These lower regression coefficients obtained in water-stressed environments indicated the increased Ol and decreased Li rates of concentration were similar to those from non-water-stressed environments within a range of 20 to 21°C in mean

Table 3. Linear regression of concentration of oleic acid, linoleic acid, linolenic acid, and oleic to linoleic plus linolenic acid ratio [Ol/(Li + Ln)], each vs. average daily mean air temperature during seed fill (Tm_{R5R7}), and precipitation minus potential evapotranspiration during the whole reproductive period (pp – PET_{R1R7}) for soybean samples from water-stressed (pp – PET_{R1R7} < 70 mm) environments (year, site, and sowing date combinations).

Dependent variable	Explanatory variable	Regression coefficient	Standard error	*P*-value	Mallow's Cp	Adjusted R^2
Oleic acid	Constant	9.1580	2.3494	0.0002		0.38
	Tm_{R5R7} (linear)	0.5668	0.1095	<0.0001	28.56	
	pp – PET_{R1R7} (linear)	–0.0113	0.0023	<0.0001	25.03	
Linoleic acid	Constant	59.0396	1.9984	<0.0001		0.29
	Tm_{R5R7} (linear)	–0.2886	0.0928	0.0024	11.58	
	pp – PET_{R1R7} (linear)	0.0100	0.0020	<0.0001	26.42	
Linolenic acid	Constant	15.2580	0.6210	<0.0001		0.70
	Tm_{R5R7} (linear)	–0.3356	0.0286	<0.0001	138.02	
	pp – PET_{R1R7} (linear)	0.0052	0.0007	<0.0001	58.78	
Ol/(Li + Ln)	Constant	0.0717	–0.0397	0.2047		0.39
	Tm_{R5R7} (linear)	0.0130	0.0026	<0.0001	26.59	
	pp – PET_{R1R7} (linear)	–0.0003	0.0001	<0.0001	30.22	

air temperature. Values were 0.34% $°C^{-1}$ at ~20°C and 0.70% $°C^{-1}$ at ~21°C for Ol, and −0.18% $°C^{-1}$ at ~20°C and −0.44% $°C^{-1}$ at ~21°C for Li. The decreased Ol/(Li + Ln) ratio rates were close to those observed within a range of 18 to 19°C in mean air temperature (0.010% $°C^{-1}$ at ~18°C and 0.020% $°C^{-1}$ at ~19°C). From these results, it is evident that in drought environments, water deficit modulates the relationship between temperature during the seed-filling period and soybean seed UFA profile. In such drought-stricken environments (inductor of stomatal closure), transpiration-limited canopies (with reduced cooling capacity) sense warmer temperatures than the air temperature recorded by meteorological stations (Carrera et al., 2009). This might explain the absence of the quadratic response of Ol, Li, and Ol/(Li + Ln) ratio to temperature found in non-water-stressed environments, thereby explaining that the fit of samples under water stress was similar to that of samples of the non-water-stressed dataset above 19°C. At temperatures above 19°C, Ol concentration and Ol/(Li + Ln) ratio increase, whereas Li concentration decreases as temperatures rise. In water-stressed environments, regression coefficients for Ol, Li, and Ol/(Li + Ln) ratio showed that Ol and Ol/(Li + Ln) ratio increased with increasing water stress (decreasing values of pp − PET_{R1R7}), whereas Li decreased (Table 3). Linolenic acid had a linear negative association with Tm_{R5R7}, with Ln concentrations being reduced with rising temperatures. It also had a linear positive association with pp − PET_{R1R7}, resulting in a decrease in Ln with increasing water deficit (Table 3). Notably, the response of UFA, including the Ol/(Li + Ln) ratio, to Tm_{R5R7} and pp − PET_{R1R7} followed the same trend, indicating that effects of both temperature and water deficit were additive in increasing UFA concentrations.

Our findings regarding response of UFA to water availability during the seed filling are in agreement with the ones of Kane et al. (1997), who reported that total rainfall during seed fill was significantly and negatively correlated with Ol and positively correlated with Li. Boydak et al. (2002) observed in field experiments that Ol and Ln were significantly affected by irrigation. Indeed, these authors observed that when the interval between irrigations was increased throughout the whole growing season, Ol concentrations increased, whereas Li concentrations decreased. In greenhouse experiments, Bellaloui et al. (2013) found that seeds harvested from water-stressed plants during filling (−90 to −100 kPa soil water potential) exhibited higher Ol and lower Li and Ln concentrations than seeds from well-watered treatments (−15 to −20 kPa soil water potential). These differences were even more pronounced when the authors analyzed seeds from plants under severe water stress conditions (−150 to −200 kPa soil water potential). Nevertheless, some studies found no significant association between UFA concentrations in soybean genotypes and irrigation or total rainfall during seed filling (Bennett et al., 2004; Kumar et al., 2006), whereas an opposite response of UFA to water availability during seed filling was reported. For example, in plants grown in greenhouse at 29°C, Dornbos and Mullen (1992) observed a decrease in Ol and an increase in Li and Ln under severe drought compared with control plants; in addition, Lee et al. (2008) found that cultivars with normal fatty acid profiles had significantly lower Ln under irrigation than under rainfed conditions across eight environments. Later, in a multiyear trial Gao et al. (2009) found that variations in soybean oil quality expressed as Ol/(Li + Ln) ratio were explained by total precipitation during the growing season, through a curvilinear model with a quadratic component. The model showed that the ratio decreased with increasing precipitation up to 305 mm, above which total precipitation as well as the ratio continued to increase. These results implied that Ol decreased with increasing precipitation up to 305 mm and then increased, whereas the opposite occurred for Li and

Ln concentrations. Even then, the biochemical bases for the responses observed by Dornbos and Mullen (1992), Lee et al. (2008), and Gao et al. (2009) are not obvious. None of these authors provided any explanation, nor did they propose possible physiological mechanisms involved in UFA changes to water availability.

Although Dornbos and Mullen (1992) evaluated the combined effect of temperature and water deficit on fatty acid profile, these authors failed to establish a relationship between drought and fatty acid composition under increasing air temperature in greenhouse experiments. To our knowledge, this is the first study conducted under field environmental conditions to demonstrate that water deficit modulates the relationship between temperature during the seed-filling period and the UFA profile of soybean seed. Little is known about the physiological bases of the response of Ol, Li, and Ln due to a combined effect of both factors. Some supporting evidence was provided in a recent research conducted by Carrera et al. (2015), who observed that soybean seeds from field plots exposed to heat and water stress during filling exhibited a lower amount of Li and Ln and a higher amount of Ol than from irrigated plots. These authors reported a significant and negative association between Ol and quantum yield of Photosystem II (ϕPSII) ($P < 0.0001$, R^2 adjusted = 0.74), whereas Ln was positively associated with both ϕPSII and total leaf chlorophyll ($P < 0.05$, R^2 adjusted = 0.77). Thus, it was concluded that the observed effect of photosynthesis (estimated through ϕPSII and total leaf chlorophyll) on fatty acid composition could be modulated by changes in assimilate supply to the seeds, since the synthesis of oil components is highly dependent on current photoassimilate production. Higher photosynthesis rates could be achieved in irrigated plots than in heat- and water-stressed ones, increasing the amount of carbon to the seeds needed for biosynthesis of fatty acids (Willms et al., 1999); this extra source of carbon could be preferentially diverted to Li and Ln, which together represent 64% of soybean oil (Wilson, 2004). Given the results from Carrera et al. (2015), it might be expected that heat and water stress could modulate UFA profile in two ways: an increase in temperature, which could affect desaturase activities involved in Li and Ln metabolism, and a reduction in the source of carbon due to the detrimental impact on photosynthesis, changing the assimilate supply to the seeds. Future studies will be required to sort out these findings. Other topics also need investigation; for instance, no study has quantified the transcripts of the genes of the key desaturases in UFA biosynthesis in soybean under heat and drought stress during seed filling, nor has the expression of genes encoding these enzymes been explored in terms of whether they are correlated with UFA accumulation under these stressed conditions.

Since environments were partially balanced in terms of cultivars to support results in Fig. 1 and Table 3, the analyses were replicated on the check cultivar DM 4800 RR ($N = 76$), which was common to all the environments involved in this study. Differences in the response of Ol, Li, Ln, and the ratio of Ol/(Li + Ln) to increasing Tm_{R5R7} between non-water-stressed and water-stressed conditions for DM 4800 are shown in Fig. 2 and Table 4, respectively. The relationships found in both types of environments (Fig. 1, Table 3) were still evident when the analysis was limited to the check cultivar. For instance, when samples were collected from non-water-stressed environments ($pp - PET_{R1R7} > 70$ mm), regression equations for Ol, Li, Ln, and Ol/(Li + Ln) ratio showed a linear significant contribution of Tm_{R5R7} (P-value, Fig. 2), explaining a high proportion of the variation (74, 76, 64, and 77%, respectively; Fig. 2). The reduced dataset also detected the quadratic response of Ol and Li to Tm_{R5R7}, with temperatures above 19°C causing an increase in Ol and a decrease in Li (Fig. 2A and 2B, respectively). As expected, the Ol/(Li + Ln) ratio also exhibited a quadratic response to temperature (Fig. 2D). Additionally, the fitted regression indicated that Ln was linearly and negatively related to Tm_{R5R7}, with a slope of $-0.43\%\ °C^{-1}$ (Fig. 2C). Furthermore, when samples were collected from water-stressed environments ($pp - PET_{R1R7} < 70$ mm), the dependence of the relationship between UFA and Tm_{R5R7} with water deficit was supported by the results from the check cultivar across all environments (Table 4). In these environments, regression equations for Ol, Li, Ln, and Ol/(Li + Ln) ratio showed a linearly significant contribution of both Tm_{R5R7} and $pp - PET_{R1R7}$ (P-value, Table 4).

The regression models found for the check cultivar DM 4800 RR were analogous to the ones obtained from the whole dataset ($N = 181$), suggesting that the large differences in UFA profile including the indicator of oil quality [Ol/(Li+Ln)] were promoted by environmental rather than genotypic effects, which is expected in multiyear and multilocation field trials (Kang, 2001). This agrees with the findings of Primomo et al. (2002), Bolognа et al. (2011), and Carrera et al. (2014b), who observed that the most important variation in Ol, Li, and Ln was mainly due to environmental effects. Although the associations between climatic variables and UFA (including the indicator of oil quality) obtained from the check cultivar dataset followed analogous trends to those from the full database, the latter allowed us to capture a wider range of climatic variability and to increase the power of the analysis by increasing the sample size.

Overall, the analysis of multienvironment field trials covering the climatic conditions during the seed-filling period allowed us to explain large proportions of the variations of Ol, Li, Ln, and Ol/(Li + Ln) ratio under both nonlimiting water availability and water stress conditions.

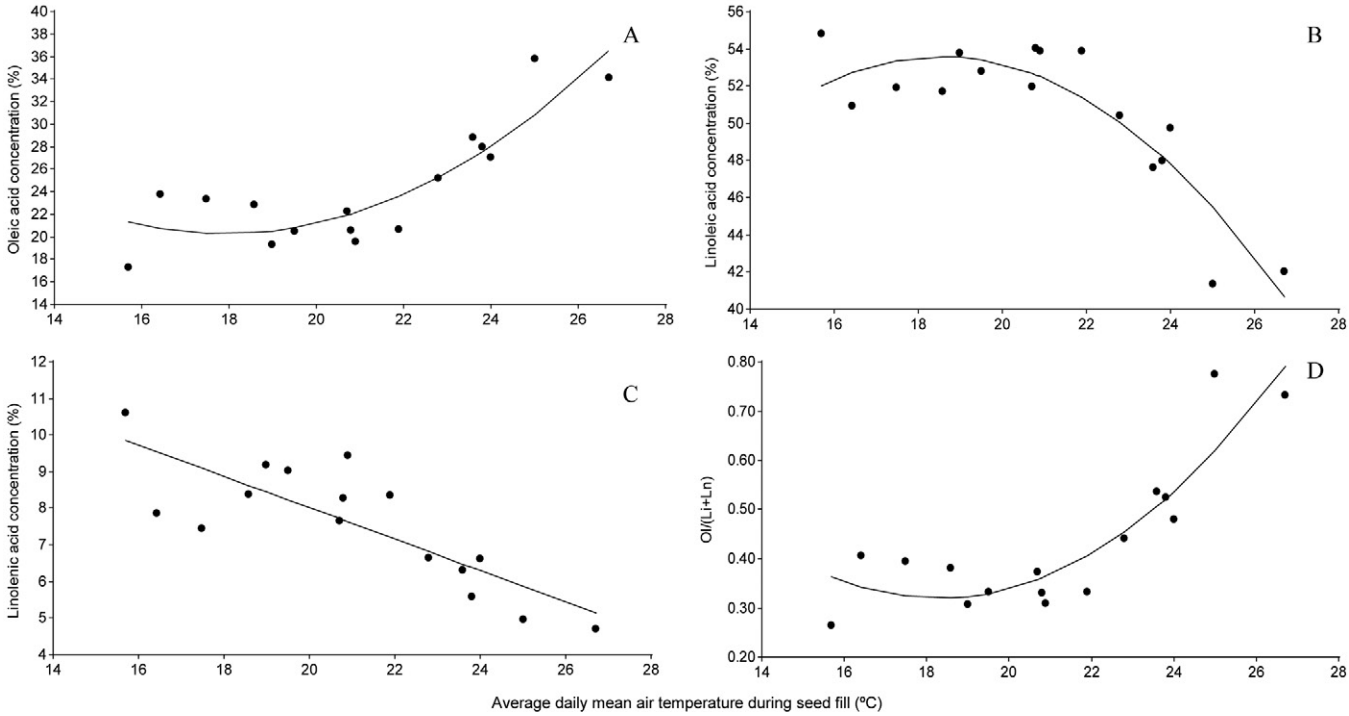

Fig. 2. Response of concentration of (A) oleic acid, (B) linoleic acid, (C) linolenic acid, and (D) oleic to linoleic plus linolenic acid ratio [Ol/(Li+Ln)] in soybean oil to average daily mean air temperature during seed fill (°C). The line represents fitted linear model for one soybean cultivar (DM 4800 RR) evaluated in all non-water-stressed (precipitation – potential evapotranspiration during the reproductive period > 70 mm $N = 16$) environments (year, site, and sowing date combinations). The following models were computed: (A) $Y = 88.47 - 7.60X + 0.21X^2$, adjusted $R^2 = 0.74$, $P_X = 0.0208$, $P_{X2} = 0.0087$; (B) $Y = -14.26 + 7.31X - 0.20X^2$, adjusted $R^2 = 0.76$, $P_X = 0.0049$, $P_{X2} = 0.0021$; (C) $Y = 16.59 - 0.43X$, adjusted $R^2 = 0.64$, $P = 0.0001$; (D) $Y = 2.54 - 0.24X + 0.01X^2$, adjusted $R^2 = 0.77$, $P_X = 0.0068$, $P_{X2} = 0.0026$.

Table 4. Linear regression of concentration of oleic acid, linoleic acid, linolenic acid, oleic to linoleic plus linolenic acid ratio [Ol/(Li + Ln)], each vs. average daily mean air temperature during seed fill (Tm_{R5R7}), and precipitation minus potential evapotranspiration during the whole reproductive period (pp – PET_{R1R7}) for soybean samples, of one soybean cultivar (DM 4800 RR) evaluated in all water-stressed (pp – PET_{R1R7} < 70 mm, $N = 60$) environments (year, site, and sowing date combinations).

Dependent variable	Explanatory variable	Regression coefficient	Standard error	*P*-value	Adjusted R^2
Oleic acid	Constant	7.5120	2.2342	0.0013	0.51
	Tm_{R5R7} (linear)	0.7045	0.1062	<0.0001	
	pp – PET_{R1R7} (linear)	–0.0062	0.0024	0.0131	
Linoleic acid	Constant	62.8372	2.5619	<0.0001	0.32
	Tm_{R5R7} (linear)	–0.5061	0.1207	0.0001	
	pp – PET_{R1R7} (linear)	0.0069	0.0026	0.0090	
Linolenic acid	Constant	14.7210	0.6774	<0.0001	0.71
	Tm_{R5R7} (linear)	–0.3259	0.0317	<0.0001	
	pp – PET_{R1R7} (linear)	0.0035	0.0007	<0.0001	
Ol/(Li + Ln)	Constant	0.0201	0.0593	0.7354	0.47
	Tm_{R5R7} (linear)	0.0167	0.0028	<0.0001	
	pp – PET_{R1R7} (linear)	–0.0002	0.0001	0.0041	

To our knowledge, the results obtained from the regression analysis showed, for the first time, that the well-established effect of temperature on UFA profile during seed development changed according to water deficit in the field.

CONCLUSIONS

In summary, this study is the first report showing the quadratic response of Ol, Li, and Ol/(Li + Ln) ratio to temperature during seed filling in environments without water restrictions. In water-stressed environments (pp − PET_{R1R7} < 70 mm), the regression coefficients linearly relating Tm_{R5R7} to Ol, Li, and Ol/(Li + Ln) ratio were significantly lower (the reductions being 92, 94, and 92%, respectively) than the analogous coefficients obtained from the analysis of samples from non-water-stressed environments. These results indicate increasing Ol and decreasing Li rates close to those observed in non-water-stressed environments within a range of 20 to 21°C mean air temperature, as well as decreasing Ol/(Li+Ln) ratio rates close to those observed within a range of 18 to 19°C mean air temperature.

Furthermore, Ln had a linear negative association with Tm_{R5R7}, with reductions in Ln concentrations with rising temperatures and a linear negative association with increasing water deficit, with both climatic factors exhibiting additive effects on UFA. Our research demonstrated that water deficit (estimated through a simple indicator, pp − PET_{R1R7}) modulates the relationship between temperature during the seed-filling period and soybean seed UFA under rainfed conditions. Therefore, our results are meaningful from a quantitative perspective, with important implications in the context of climate change.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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