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Soybean (*Glycine max* L. Merr.) seed composition response to soil flooding stress

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Abstract

Soybean is a valuable commodity due to its high oil and protein content and its nutritional and functional food value. Changes in soybean seed composition by environmental stressors including heat and drought have been documented, but the effects of flooding are not yet known. This study profiles the change in seed composition of five soybean plant introductions (PIs) tolerant to flooding stress and the flooding sensitive check cultivar Williams in three environments. The results showed that flooding did not significantly affect seed protein, oil or palmitic acid, but increased oleic and stearic acid levels in all genotypes. However, the linoleic and linolenic acids, daidzein, genistein, and glycitein contents were significantly reduced in all genotypes. The effects of environment were significant for most seed composition traits under flooding stress, except palmitic acid, linolenic acid and glycetin. Single degree of freedom orthogonal comparison showed that seed quality index (SQI) - a composite indicator of seed quality - increased by 4% in the PIs, but decreased by 5% in the check cultivar. The results suggested that SQI is a useful criterion when genetic selection for seed composition is to be made in different environments. Given the more frequent extreme weather patterns possible with global climate change, caution is needed in the selection of environments for testing and producing soybeans for special composition traits.

Key words: Abiotic stress, environmental stress, flooding, flooding response index, *Glycine max*, isoflavone, seed composition, seed quality index, soybean, waterlogging.

Introduction

Soybean (*Glycine max* [L.] Merr.) is one of the most important crops in the world. Produced for their high nutritive value, soybean seeds average 421 g kg^{-1} protein, 195 g kg^{-1} oil, 300 g kg^{-1} carbohydrates of the total seed dry mass [1, 2]. Soybean seeds also contain mineral nutrients including Fe, Cu, Mn, Ca, Mg, Zn, Co, P and K and vitamins B1, B2 and B6, as well as isoflavones [3, 4]. The composition of soybean seeds depends on several factors, including genotype, geographic location, growing season weather condition, soil condition, maturity, and agronomic practices [5-8].

Studies have shown changes in seed composition due to environmental stresses, especially drought and temperature [2, 9, 10]. The response of protein concentration to air temperature during seed development showed a two-phase pattern with the lowest concentration at a mean temperature of 22°C. As the mean temperature decreased from 22 to 14°C, protein concentration increased. Protein concentration also increased as the mean temperature increased from 22°C to 28°C [5]. Oil concentration, however, increased linearly with the increase in mean temperature up to 28°C during the reproductive stage. The average increase in oil concentration across different genotypes and environments was about 6.9 g kg^{-1} °C^{-1} and ranged from 5.2 to 8.5 g kg^{-1} °C^{-1} [11, 12]. Protein plus oil content appear to increase linearly with increasing mean air temperature.

Temperature also affects fatty acid profile. Seed development at higher temperatures results in significant increase in oleic acid while linoleic acid and linolenic acid decrease [10, 13-17].

Oleic acid, the 18-carbon monounsaturated fatty acid, is a precursor to linoleic and linolenic acids. The relationship between oleic acid and linoleic/linolenic fatty acids is direct and negative. Increased seed oleic acid content is accompanied by a highly correlated decrease in seed linoleic and linolenic acid content [18]. Oleic acid is less reactive with oxygen and therefore significantly more stable than linoleic and linolenic acid. Linolenic acid is responsible for the oxidative instability and flavor problems in soybean oil [19]. Reducing the amount of linoleic and linolenic acid in soybean is a desired breeding objective so that oxidatively stable soybean oil can be produced without the production of trans-fatty acids [20]. Higher oleic acid level is also desirable because of its potential health benefits with better nutritional and functional attributes [21].

In addition to high oleic acid levels, isoflavones have also been associated with the health benefits of soybean food products. Research on isoflavones has indicated several important biochemical, biological and biomedical properties including anti-oxidant activity [22-24], antifungal activity [25], antitumor activity [26], and estrogenic properties [27].

Isoflavone contents of soybean seeds also change with genotypes, locations, and temperatures during seed development. The total isoflavones of soybean seeds varied from 116 to 309 mg g^{-1} across varieties [28]. When the same variety was grown at different locations, isoflavone concentrations varied from 46 to 195 mg g^{-1}. Isoflavone concentration also changed from year to year when soybeans were grown in the same location. Management practices have been shown to affect isoflavone content. Early planting resulted in 1.3-fold increase and irrigation enhanced the isoflavone content of both early- and late-planted soybeans as much as 2.5-fold [29].

It has been reported that high temperatures during seed development significantly reduced concentrations of isoflavones in soybean seeds; while lower temperature increased their isoflavone concentrations several-fold [30]. It is therefore suggested that to increase the concentration of isoflavones, soybean should be grown in localities that have lower temperatures during grain filling period [31].

Flooding is the second most damaging environmental stressor on crop growth, after drought, and affects about 16% of the production areas worldwide [32]. During the period from 1951 to 1998, average crop losses due to flooding in the U.S. were reported to be around 3% per year or worth ~$1.5 billion a year (USDA National Agricultural Statistics, 2000). Given the current trend in global climate change with more extreme weather patterns, the National Aeronautics and Space Administration simulation models predicted that flooding damage to crops in the U.S. will double to ~$3 billion per year by the year 2030 [33]. The effects of soil flooding on soybean seed composition traits such as protein, oil, fatty acids and isoflavones have not been reported. The objective of this research was to examine the effects of soil flooding stress on seed composition of five soybean plant introductions tolerant to flooding stress and the check cultivar Williams.

Materials and Methods

Field experiment: Field experiments were conducted in three environments: Delta Center, University of Missouri, Portageville (36°25'N and 89°20'W), Missouri, USA in 2007 and 2008 (DEC07 and DEC08, respectively) and the Horticulture & Agro-Forestry Center, Missouri Agricultural Experiment Station, Columbia (39° 02'N; 92°75'W), Missouri in 2007 (COL07). The soil at Portageville is a Sharkey clay (very fine, smectitic, thermic Chromic Epiaquerts). At the Columbia farm, the soil is a Mexico silt loam (fine, smectitic, mesic Aeric Vertic Epiaqualfs).

Five plant introductions (PI086449-0, PI398395, PI416753, PI423838, and PI567251) from the USDA Soybean Germplasm Collection (Urbana, Illinois) known for their tolerance to flooding stress [34] and the check cultivar Williams were used in this study. Seeds were planted in hill plots at 10 seeds per hill, spaced 60 cm apart within rows and 76 cm between rows. Planting dates were 7 May, 30 May and 23 May for DEC07, DEC08 and COL07, respectively. At all locations, the experiment was conducted on two adjacent fields. The non-flooded control field had drainage ditches around and between replications, while the flooded field was surrounded by dikes to control the water level. Within each treatment (flooding vs. control), the genotypes were assigned randomly with four replicates. Flooding treatment was imposed when the plants were at the R2 reproductive growth stage [35] by pumping the water to about 5 to 10 cm above the soil surface. Flooding continued until the plants showed symptoms of injury (7 days). During the flooding period, additional water was added to maintain the water levels. At the end of the flooding treatment, the dike was open to allow the field to drain. At the end of the growing season, seeds were harvested, dried and analyzed for chemical composition.

Total oil and protein content: A seed sample (20 g) was analyzed for protein and oil content using an Infratec 1255 NIR Food and Feed Grain Analyzer (Ultra Tec Manufacturing Inc., Santa Ana, CA). The Infratec analyzer is a near infrared transmittance instrument used for simultaneous constituents determination of whole grains [2].

Fatty acid profile: Fatty acids were analyzed as described by Wilson *et al*. [36]. Each seed sample (1 g) was manually crushed to powder with a hammer and extracted in 5 mL chloroform: hexane:methanol mixture (8:5:2 by v/v/v) overnight. Derivatization was done by transferring 100 µL of extract to a vial and adding 75 µL of methylating reagent (0.50 M methanolic sodium methoxide:petroleum ether: ethyl ether, 1:5:2 by v/v/v). Hexane was added to dilute samples to 1 mL. An Agilent (Palo Alto, CA) series 6890 capillary gas chromatograph® fitted with a flame ionization detector (275°C) was used with a DB-23 capillary column (Alltech Associates, Deerfield, IL). Standard fatty acid mixtures (Animal and Vegetable Oil Reference Mixture 6, AOACS) were used for calibration.

Isoflavone extraction and analysis: Each seed sample (1 g) was ground to a fine powder using a seed grinder (model 5XBG008, General Electric, New York). The powder was extracted with 5 mL of 80% (v/v) methanol at 55°C. After centrifugation at 5000 g for 5 min, the supernatant was filtered through a 0.45 µm PTFE nylon syringe filters (Fisher Scientific®). Samples were analyzed by reverse-phase HPLC equipped with a photodiode array detector (Beckman Coulter, Fullerton, CA) [37]. Separation and elution were accomplished using an 18-min linear gradient initiated with a solution of 20% (v/v) methanol and 80% 10 mM ammonium acetate (pH 5.6) and completed with 100% methanol at the flow rate of 1 mL min^{-1}. Identification and quantification of each isoflavone component were based on available standards (Indofine Chemical Co., Somerville, NJ). The analysis was repeated four times for each plot and the mean of each experimental unit was computed for further statistical analysis.

Calculation of seed quality index: Soybean seed quality index (SQI) was calculated based on protein, oil, oleic acid, linoleic acid, linolenic acid, daidzein, genistein, and glycitein concentration by modifying the additive approach [38]. The approach based on data normalization considering "higher values of protein, oil, oleic acid, daidzein, genistein, and glycitein ($x\ x_{max}^{-1}$) and lower values of linoleic acid and linolenic acid [$1 - (x\ x_{max}^{-1})$] is a better indicator of soybean seed quality" followed by summation and averaging of data to calculate the index. Datum (x) for each individual property (x_i) of soybean seed was normalized to transform the values into > 0 to 100 scores in the datasets, $x_i = (x\ x_{max}^{-1})$ or $x_i = [1 - (x\ x_{max}^{-1})]$. Data were normalized for reducing heterogeneous variances of the error and to simplify the relationship between random error influenced variables. The normalized scores ($x_i + \ldots + x_n$) were

then averaged to calculate the SQI =$\Sigma(x_i + ... + x_n)\ n^{-1}$. Thus, SQI could range from > 0 to ≤ 100 with 100 being excellent in quality and 0 being extremely poor in quality.

Statistical analysis: Analysis of variance was conducted using PROC ANOVA of SAS® PC for Windows Version 9.1.3 (SAS Institute Inc., Cary, NC) to determine the effects of treatment, genotype and environment on seed composition traits. A dependent variable "*flooding response index*" was calculated by dividing the flooded seed composition by the control composition. The ratios of flooding/control were transformed to $\log_2$ format in order to depict the symmetrical distribution of the positive and negative responses around the base line of $\log_2 = 1$ where no changes in seed composition due to flooding stress occurred [39]. Analysis of variance was conducted on the $\log_2$ data using the same procedure as described above. The TMEV module of the TM4 software (http://www.tm4.org) was used to develop a "heat map" for quantitative visualization of the *flooding response* of seed composition in three environments.

Results and Discussion

Effects of flooding on soybean seed composition traits: Response of seed composition traits to flooding stress was tested in this study of five plant introductions, PI086449-0, PI398395, PI416753, PI423838, and PI567251 and the check cultivar Williams. Analysis of variance indicated that flooding at the early reproductive R2 stage did not affect the levels of protein and oil content of the six genotypes (Table 1). The results were contrary to those from drought stress where it has been reported to increase in protein content by 4.4% and decrease in oil content by 2.9% [10]. The sample of five plant introductions and one check cultivar used in this study was sufficient to indicate that the differential response of soybeans to flooding and drought stress was probably not genotype-related. However, further testing will be needed to confirm if flooding could have initiated a different set of abiotic stress responses in seed composition than drought.

The average protein and oil content at 13% seed moisture, across genotypes, treatments, and environments were 370 and 190 g kg^{-1}, respectively. Williams, the check cultivar, contains lower protein and higher oil than the five plant introductions tested (Table 2). The average palmitic, stearic, oleic, linoleic, and linolenic acid of the control treatment across genotypes were 113, 34, 247, 522, and 71 g kg^{-1} of total oil, respectively. These concentrations were within the reported ranges of 100 to 120 g kg^{-1} for palmitic acid, 22 to 72 g kg^{-1} for stearic acid, 203 to 453 g kg^{-1} for oleic acid, 281 to 561 g kg^{-1} for linoleic acid, and 19 to 80 g kg^{-1} for linolenic acid in soybean oil [8, 40, 41]. Flooding did not significantly change the concentration of palmitic acid (113 g kg^{-1}), but increased the concentration of stearic acid and oleic acid to 37 and 272 g kg^{-1}, respectively. The concentration of linoleic acid, and linolenic acid were reduced to 497 and 64 g kg^{-1}, respectively by flooding (Tables 1 and 2). The negative correlation between oleic acid and linoleic acid/linolenic acid has been documented in the literature [18]. Similar results of increasing oleic acid and reducing linoleic and linolenic concentration have also been reported for high temperature stress[2, 10, 13, 14, 15, 40]. The conversion of oleic acid to linoleic and linolenic acids is catalyzed mainly by the two enzymes ω-6 desaturase and ω-3 desaturase. To explain the high concentration of oleic acid at high temperature, it has been suggested that these desaturase enzymes have optimum activity at low temperature (18/14°C day/night) [42]. More related to flooding stress was the hypothesis that desaturase activity was mediated by oxygen in the cell cytoplasm, which is higher at low temperature [43]. The exact biological mechanism(s) that regulate the increase in oleic acid by flooding and temperature remains unknown. Soybean breeders, therefore, need to be concerned about the effects of environmental stresses, including heat and flooding, when screening germplasm and breeding lines for high oleic acid content.

The average concentration of daidzein, genistein, and glycitein in the control treatment was 132, 286, and 26 mg kg^{-1}, respectively (Table 2). Flooding reduced the concentration of these isoflavones to 106, 200 and 19 mg kg^{-1}, respectively. Because of their potential health benefits as functional and nutritional food, research in the past decade has focused on improving the concentration of isoflavones in soybean seed [6, 29, 31, 45-48]. Isoflavones are a group of defense compounds. In leaves and roots, they increase in response

Table 1. Analysis of variance to determine the effects of genotype, treatment, environment and their interaction on the composition of soybean seed from experiments conducted at Columbia, MO in 2007 and Delta Center, Portageville, MO in 2007 and 2008.

Source of variation	DF	Protein	Oil	Palmitic	Stearic	Oleic	Linoleic	Linolenic	Daidzein	Genistein	Glycitein
		Mean Square									
Genotype (GEN)	5	34.22***	12.87***	13.11***	3.39***	271.88***	343.82***	15.12***	48286***	281146***	2946***
Treatment (TRT)	1	24.09	4.76	0.71	2.52***	629.33***	233.98**	15.09***	24767***	262929***	1356***
Environment (ENV)	2	24.48*	17.98***	19.05***	2.62***	41.23*	39.22	7.28***	49828***	26262***	12196***
GEN*TRT	5	10.31	3.68	0.51	0.12	30.56*	3.13	0.22	7570***	20540***	486***
GEN* ENV	10	10.2	4.98*	1.47	0.14	70.20***	52.96***	1.51	9007***	6489***	874***
TRT* ENV	2	14.66	0.58	0.35	1.04***	92.42***	10.4	1.1	1400***	3346**	483***
Orthogonal comparison											
Orthogonal (Ortho)	1	35.12	27.13**	4.82	11.08***	395.33***	453.25***	1.4	55675***	233259***	2208*
Ortho * TRT	2	13.14	5.55	0.35	1.26***	321.74***	117.03*	7.59**	25343***	163395***	689

*, **, *** Significant (F-test) at the 0.05, 0.01 and 0.001 levels respectively.

Table 2. Main effect means of flooding treatment, genotype and orthogonal comparison of soybean seed composition traits from experiments conducted at Columbia, MO in 2007 and Delta Center, Portageville, MO in 2007 and 2008.

Genotype	Protein		Oil		Palmitic		Stearic		Oleic		Lenoleic		Lenolenic		Daidzein		Genistein		Glycitein	
	g kg^{-1}						g kg^{-1} of total oil										mg kg^{-1}			
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Control																				
PI086449-0	381	3.3	189	1.3	110	1.8	37	0.6	266	11.6	524	11.1	62	5.7	63	10	107	12	13	3
PI398395	364	2.5	177	1.2	124	1.5	31	0.7	226	3.7	536	3.6	82	1.4	149	10	252	7	24	4
PI416753	337	3.1	172	1.6	99	0.9	31	0.3	283	2.8	443	4.1	60	6.0	138	12	385	21	21	3
PI423838	382	1.0	186	0.9	116	2.4	31	0.8	247	6.1	536	4.8	70	1.8	47	7	76	6	8	1
PI567251	375	1.5	180	1.1	117	1.9	33	0.4	242	5.7	531	4.7	78	1.8	112	12	175	10	16	3
Williams	351	1.5	198	0.7	108	1.4	40	0.5	217	4.2	562	3.3	72	1.0	160	4	359	5	30	3
Average	*365*	*2.1*	*184*	*1.1*	*113*	*1.6*	*34*	*0.5*	*247*	*5.7*	*522*	*5.3*	*71*	*2.9*	*132*	*9*	*286*	*10*	*26*	*3*
Flooding																				
PI086449-0	377	2.8	196	1.7	111	2.0	39	0.5	297	7.4	498	6.3	56	1.2	160	8	354	8	48	8
PI398395	369	4.0	176	1.8	125	2.1	33	0.8	259	3.9	510	4.2	74	1.4	76	5	197	11	14	3
PI416753	369	2.2	189	2.3	106	1.4	36	1.6	270	9.0	431	6.5	57	2.6	206	25	423	17	35	8
PI423838	381	1.8	188	2.7	116	1.7	33	1.0	279	6.7	510	5.9	62	1.9	130	9	231	11	29	5
PI567251	379	1.1	181	2.5	116	1.9	36	1.4	283	11.2	496	8.5	69	2.4	66	4	117	3	8	1
Williams	365	3.1	192	2.4	110	1.5	43	1.0	245	8.7	536	7.4	67	1.3	120	18	244	14	28	7
Average	*373*	*2.5*	*187*	*2.2*	*114*	*1.8*	*37*	*1.0*	*272*	*7.8*	*497*	*6.5*	*64*	*1.8*	*106*	*12*	*200*	*11*	*19*	*5*
Orthogonal comparison																				
Control																				
PIs	368	2.4	181	3.2	113	2.2	33	0.7	254	5.2	523	4.0	40	1.7	117	3	259	14	24	3
Check	352	1.5	198	0.7	108	1.4	40	0.5	217	4.2	562	3.3	33	0.9	206	6	423	17	35	8
Flooding																				
PIs	375	1.3	186	1.3	115	1.1	35	0.6	298	6.8	489	5.6	56	1.3	103	2	192	13	18	2
Check	366	3.1	192	2.4	110	1.5	43	1.0	245	8.7	536	7.4	74	1.4	120	2	244	14	28	7

to biotic and abiotic stresses, such as under fungal attack and UV irradiation [49]. At the same time, the biosynthesis of isoflavones is energy intensive and requires a substantial amount of reducing power of the cell in the form of NADH and NADPH (for the biosynthesis of malonyl-CoA and p-coumaroyl CoA). It is plausible that under flooding stress, the limited reducing potential is re-directed toward oleic acid biosynthesis rather than isoflavone biosynthesis.

Single-degree of freedom orthogonal analysis was used to compare the seed composition response of the PIs and the check cultivar Williams to flooding stress. All the seed composition traits, except protein, palmitic and linolenic acid, were significantly different ($p \leq 0.05$) between the PIs and the check cultivar Williams. The interaction of orthogonal comparison x treatment was also significant for all traits except protein, oil, palmitic acid and glycetin (Table 1). While the PIs were more tolerant to field flooding than Williams, whether the results reflect the differential response due to flooding tolerance will require additional research.

Effects of genotype on soybean seed composition in response to flooding stress: Analysis of variance indicated that seed composition traits were significantly different among the genotypes (Table 1). The mean and standard error of seed composition of each genotype under control and flooded conditions are reported in Table 2. To gain insight into the effects of flooding on soybean seed composition, a dependent variable "*flooding response index*" (FRI) was calculated by dividing the flooded seed composition trait by the control seed composition (F/C). When a seed composition trait is induced by flooding, its FRI is >1 (positive effect of flooding)). Alternatively, when a seed composition trait is suppressed by flooding, its FRI is <1 but >0 (negative effect of flooding). To better depict the positive and negative effects of flooding on seed composition, the FRI of each seed composition trait was normalized to $\log_2$ (flooding/control). Analysis of variance of the $\log_2$ FRI indicated that changes in protein, oil and isoflavones were highly affected by genotype ($p \leq 0.001$) (Table 3). Among the fatty acids, changes in linolenic acid ($p \leq 0.05$) were also affected by genotype.

The response matrix (heat map) showing the $\log_2$ flooding response index of seed composition in the six genotypes tested at three environments was presented in Fig. 1. The general pattern of positive (red color) and negative (green color) changes in seed composition in response to flooding was similar among the genotypes. Flooding significantly increased the concentration of stearic, oleic, linoleic and linolenic acid but reduced isoflavones concentrations in all genotypes (Table 2 and Fig. 1).

Effects of environment on soybean seed composition in response to flooding stress: Analysis of variance of the $\log_2$ FRI indicated that the effects of environment were significant for protein ($p \leq 0.05$), highly significant ($p \leq 0.001$) for oil, stearic acid, oleic acid, linoleic acid and the isoflavones (Table 3). The statistical significance of individual seed composition traits in each of the three environments as indicated by DMRT ($p \leq 0.05$) is shown in Fig. 1. Flooding showed the strongest positive effects on oleic acid content. The average changes were 60 g kg^{-1} at the DEC08 environment, 90 g kg^{-1} at the DEC07 environment and 440 g kg^{-1} at the COL07 environment.

Table 3. Analysis of variance to determine the effects of genotype, environment and their interaction on the $\log_2$ FRI (Flooding Response Index) of soybean seed composition and SQI (Seed Quality Index) from experiments conducted at Columbia, MO in 2007 and Delta Center, Portageville, MO in 2007 and 2008. FRI and SQI were calculated as described in the text.

		Mean Square and F test										
Source of variation	DF	Protein	Oil	Palmitic	Stearic	Oleic	Linoleic	Linolenic	Daidzein	Genistein	Glycitein	SQI
Genotype (Gen)	5	0.0069*	0.0115***	ns	ns	ns	0.0191*	ns	1.6001***	0.8135***	2.5041***	0.0452***
Environment (Er	2	0.0024*	0.0080***	ns	0.1277***	0.2991***	0.0606***	ns	0.8508***	0.3099***	ns	0.0698***
Gen*Env	10	0.0017*	0.0082***	ns	ns	0.1141***	0.0346***	ns	0.7577***	0.4821***	0.9497***	0.221***
*, **, *** Significant (F-test) at the 0.05, 0.01 and 0.001 levels respectively.												

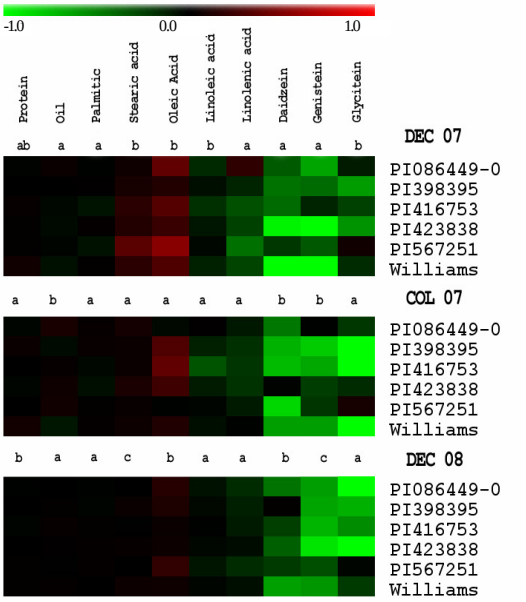

Figure 1. Response matrix of seed composition traits of six soybean genotypes to soil flooding in three environments — Delta Center 2007 (DEC07), Columbia 2007 (COL07), and Delta Center 2008 (DEC08). The red color indicates the trait was induced by flooding. The green color indicates suppression. The scale above the response matrix shows the $\log_2$ (flooding/control). The seed composition of each environment with the same letter is not different at $p<0.05$ as indicated by DMRT.

According to Wilson [2], oleic acid levels in soybean seed were 350 to 820 g kg^{-1} higher, dependent on genotype, when grown at 30°C compared to 18°C. The effects of environment on response of oleic acid to flooding in this study were probably not related to temperature since the average, average maximum and average minimum temperatures during the reproductive period of the three environments only differed by ± 1°C (data not shown). Stearic acid was also positively influenced by flooding stress with an average increase of 20 g kg^{-1} at the DEC08 environment, 60 g kg^{-1} at the DEC07 environment and 320 g kg^{-1} at the COL07 environment. However, flooding reduced seed linoleic and linolenic acid contents across the three environments by an average of 20 and 60 g kg^{-1}, respectively. The environment x genotype interaction was significant for the three isoflavones (Table 3). The large variability by year and by location of isoflavone content has been documented in the literature [29, 50]. However, the general pattern of flooding response of isoflavones across the six soybean genotypes under three environments in this study was quite consistent (Fig. 1).

Effects of flooding on seed quality index: The quality, utility and economic worth of soybean have traditionally been determined by its protein and oil content [2]. Recent interest in the potential health beneficial effects of oleic acid and isoflavones has led to the use of soybean in functional foods and nutraceuticals. High-oleic and high-isoflavone soybean can command a premium price and is becoming a new-value added niche market for soybean producers. To quantify seed quality across genotype, treatment and environment, the composite seed quality index (SQI) was computed based not only on protein and oil concentration, but also on oleic acid, linoleic acid, linolenic acid, daidzein, genistein, and glycitein concentration by the modifying additive approach [38]. The average SQI of the six genotypes in the control treatment was 52%. Flooding increased the SQI to 54%. The difference although very small, was significant at $p \leq 0.05$. This increased SQI due to flooding reflected the increase in oleic acid and decrease in linoleic and linolenic acids. Analysis of variance indicated that $\log_2$ flooding response of SQI was affected by genotype, environment and the genotype x environment interaction (Table 3). The SQI of the six genotypes under control and flooding conditions was presented in Fig. 2. In response to flooding, SQI either remained unchanged in PI398395, PI423838 or increased in PI086449-0, PI416753 and PI567251 at 5, 6 and 8 %, respectively. However, flooding reduced the SQI of the check cultivar Williams by 4% (Fig. 2A). Orthogonal analysis showed the differential response of SQI of the check cultivar Williams compared to the PIs (Fig. 2B).

Conclusions

The results represent new findings on the response of soybean seed composition traits to flooding stress. Flooding stress did not affect protein, oil and palmitic acid concentration, but increased the concentration of stearic and oleic acid, while it reduced the concentration of linoleic and linolenic acids, daidzein, genistein, and glycitein in soybean seeds. Seed quality index (SQI), the single composite integrator of seed quality based on protein, oil, oleic acid, linoleic acid, linolenic acid, and isoflavones content increased in the plant introductions in response to flooding stress, but decreased in the check cultivar Williams. Further testing will be needed to confirm if the differential response of the two groups may be related to the flooding tolerance of the plant introductions. The results provide information important in modeling soybean

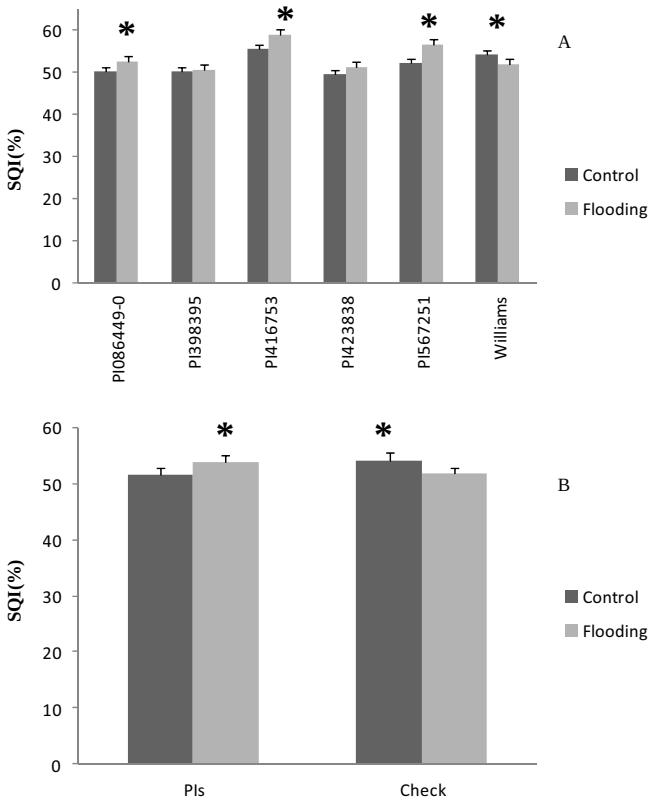

Figure 2. Seed Quality Index (SQI). A. Change in SQI of the six genotypes due to flooding stress. B. Orthogonal comparison of SQI of the PIs and check. SQI was calculated as described in the text. The PIs were composed of the five plant introductions (PI086449-0, PI398395, PI416753, PI423838, and PI567251) that showed more tolerant to flooding than the check cultivar Williams. Means with "$_*$" were significantly different between control and flooding treatments at $p \leq 0.05$.

seed composition traits under flooding, a stress condition predicted to be more prevalent in the future by global climate change.

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