GENOTYPE, ENVIRONMENT AND GE INTERACTION EFFECT ON SOYBEAN OIL COMPOSITION

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By

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The undersigned, appointed by the Dean of the Graduate School, have examined the thesis entitled:

GENOTYPE, ENVIRONMENT AND GE INTERACTION

EFFECT ON SOYBEAN OIL COMPOSITION

Presented by Martin L. Oliva

A candidate for the degree of Master of Science

And hereby certify that in their opinion it is worthy of acceptance.

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Literature Review

Plant breeding objectives have traditionally been focused on increasing crop yields. In recent years, however, improving chemical composition of seed through conventional breeding, mutation breeding or biotechnology has become a major goal in the breeding of many agricultural commodities (Yadav, 1996). In soybean, research on improving seed composition has focused on increasing protein and oil content, improving the amino acid balance of the protein, lowering phytic acid content, increasing isoflavone content and modifying fatty acid composition of the oil (Wilson, 2004).

Modifying fatty acid composition of soybean seed oil will foster new uses, improve functionality, reduce processing costs and enhance nutritional value of the soybean, among other benefits. Several soybean genotypes that produce novel fatty acid profiles have been developed through mutation breeding or transgenic approaches. Evaluation of agronomic traits and the stability of the oil profile of these genotypes across environments is necessary to determine their utility in plant breeding programs to develop soybean cultivars with enhanced oil quality (Primomo et al., 2002).

Relationship between Modified Fatty Acid Traits and Agronomic Traits

Several studies have been conducted to evaluate the association among lines with different fatty acid profiles for yield and other agronomic traits.

Association of reduced linolenate and other traits

Soybean oil with low linolenic acid (18:3) has oxidative stability superior to that of conventional soybean which may diminish the need for partially hydrogenating the oil for food uses. Recessive alleles at the *Fan1*, *Fan2* and *Fan3* loci have been determined to be additive in lowering 18:3 from 8% to 1% (Wilson, 2004).

C1640 is a low linolenate line developed by mutation breeding. Its low linolenate content was found to be conditioned by a recessive allele at the *Fan* locus (Wilcox and Cavins, 1985). Wilcox et al. (1993) found no association between the low linolenic acid trait from C1640 (*fan fan* alleles) and seed yield, plant maturity, lodging or height. They concluded that the low yield of C1640 may be controlled by genetic changes independent of the mutation for low 18:3. Therefore, developing agronomically acceptable, high yielding cultivars with low linolenic acid should be possible by incorporating the *fan fan* alleles into adapted cultivars.

Walker et al. (1998) tested three populations segregating for *fan1* and *fan2* alleles. They obtained no significant difference in mean yield between reduced linolenic acid and normal linolenic acid progenies in two of the three populations. In the third population the mean yield of the progenies with low linolenic acid content was significantly lower than the mean yield of the normal linolenic progenies. However, maturity, lodging and height were not significantly associated with the low linolenic acid trait among the three populations studied. They concluded differences in the yield response of the populations may be due

to genetic background of the parents since each cross with the normal parent involved a different reduced 18:3 parent.

Ross et al. (2000) tested agronomic and seed traits of soybean lines containing 1% linolenate derived from A29 (*fan1fan1*, *fan2fan2*, *fan3fan3* alleles) in three backcrossed populations with three high yielding low linolenic acid *fan1fan1*, *fan2fan2* recurrent parents. They found that mean seed yield of the 1% linolenate lines was not significantly different from the 2% linolenate lines in two populations, but was significantly lower in the third. They also found slight and inconsistent differences in maturity, lodging and plant height, but the ranges among lines were similar for the three populations. Results from these studies provide evidence that there is no pleiotropic or negative linkage between the low linolenate trait and agronomic traits.

Association of reduced palmitate and other traits

Reducing saturated fat (% palmitate and % stearate) in soybean oil is important for improving health benefits to humans. Soybean oil in conventional soybeans contains about 15% saturates. New labeling laws will require saturated fats in soybean oil to be reduced from 15% to 7% or less in order for food to be labeled low in saturated fat (Wilson, 2004). Saturated fat from soybean oil, especially palmitate, has been determined to increase risk of arteriosclerosis and heart disease. Conventional soybeans contain about 10 to 11% palmitic acid. Major recessive genes at the *Fap* loci and minor genes can lower palmitic acid content from 10% to 4% or less (Wilson, 2004).

Ndzana et al. (1994) developed soybean populations by crossing reduced palmitate lines with *fap1fap1*, *fap3fap3* alleles and high yielding cultivars having normal palmitate content. They found significantly lower mean seed yield ($p<0.05$) in the reduced palmitate lines compared to lines with normal palmitic acid in two of the three populations. Low palmitate lines in the third population had lower mean yield than normal lines but differences were not statistically significant ($p<0.05$). When data for reduced and normal palmitate lines were combined palmitate content and seed yield were significantly correlated for the three populations. Associations of other agronomic characteristics and chemical composition with palmitic acid were inconsistent, with the exception of oil content which was significantly lower in the low palmitate lines in the three populations. They proposed pleiotropic effects or the possible linkage of the *fap1* and *fap3* alleles with genes affecting agronomic and seed traits.

Rebetzke et al. (1998) found a significant effect of a major gene from N87-2122-4 for reduced palmitic acid on seed yield, plant height, seed oil content, oleic and linolenic acid contents in two soybean populations. Mean seed yield of low palmitate lines was about 11% below normal palmitate lines. Minor genes conditioning palmitate content showed no effect on seed yield. Evidence suggests that the major gene conditioning reduced palmitate may be linked with genes that result in low seed yield. They suggested that high yielding, low palmitate lines can be derived by selection in large populations segregating for both traits (Rebetzke et al., 1998).

Association of high palmitate and other traits

Hartmann et al. (1996) studied the association of the *fap2-bfap2-b*, *fap4fap4* genotypes having a palmitate content of 250 g kg^{-1} of oil with agronomic and seed traits of soybean. Seed yield of elevated-palmitate lines was 257 kg ha^{-1} and 209 kg ha^{-1} less than normal-palmitate lines in the two populations they studied. In one population the difference in seed yield between the highest yielding normal-palmitate line and elevated-palmitate line was 408 kg ha^{-1} while in the other population the difference was only 96 kg ha^{-1}. They concluded, however, it should be possible to obtain high yielding cultivars with elevated-palmitate content. Elevated-palmitate lines had significantly lower protein and oil content than normal-palmitate lines. Stearic, oleic and linoleic acid contents were significantly lower in the elevated-palmitate lines while mean linolenic acid content was significantly higher. The negative influence of elevated palmitate on seed yield, protein and oil content may be caused by linkage or pleiotropic effects of the *fap2-b* and *fap4* alleles with genes that cause undesirable physiological effects (Hartmann et al., 1996).

Hayes et al. (2002) studied the association of elevated-palmitic acid alleles with agronomic and seed traits in three populations derived from parents with about 260 g kg^{-1} and about 440 g kg^{-1} palmitate content. The parents with 260 g kg^{-1} palmitate had the *fap2-b* and *fap4* alleles and the parents with the 440 g kg^{-1} palmitate supposedly had these alleles plus at least two other unknown alleles for high palmitate. Most results were similar to those obtained by Hartmann et al. (1996). Yields were significantly lower in the high palmitate (>400

g kg^{-1}) lines in two of the three populations studied. The high palmitate lines yielded 36.5% to 41.8% less seed weight, had 16 g kg^{-1} less seed oil content and significantly smaller seed in the three populations. Protein content in lines with >400 g kg^{-1} palmitate averaged 9 g kg^{-1} more than lower palmitate lines which is contrary to results reported by Hartmann et al. (1996). Hayes et al. (2002) also found higher palmitate lines were significantly lower in mean oleic and linoleic acid content than normal palmitate lines, but the variation within these lines was sufficient to allow selection for oleic and linoleic acid content when developing cultivars with >400 g kg^{-1} palmitate (Hayes et al., 2002).

Environmental Effect on Fatty Acid Composition

Environmental influence on the fatty acid profile of soybean oil has been addressed in many studies. Howell and Collins (1957) found linoleic and, especially, linolenic acids in seeds of cultivars were negatively correlated with temperature in field and greenhouse studies. Wolf et al. (1982), reported seed development at higher temperatures resulted in a significant decrease in linoleic and linolenic acid contents, and a significant increase in oleic acid content. Palmitate and stearate contents generally were unaffected by changes in temperature. Similar results with regard to the effect of temperatures on the content of oleic, linoleic and linolenic acids were obtained by Cherry et al. (1985) in a field experiment grown in a northern (Indiana) and a southern (Mississippi) location. Carver et al. (1986) also reported similar findings in field studies consisting of eight environments in a single year. Cherry et al. (1985) also

reported significant differences between locations in levels of palmitic acid in five of six genotypes tested and in levels of stearic acid in three of six genotypes tested. There was a significant G x E interaction for stearic and linoleic acid contents.

Wilcox and Cavins (1992) studied the response of fatty acid composition to planting dates for the cultivar ‘Century’ and two low linolenic acid soybean lines (C1640 and 9509) in Indiana across years. Planting date had a significant effect on fatty acid profile in three of the five years studied. Levels of palmitic acid decreased slightly and levels of stearic increased slightly with successively later planting dates. Linolenic acid content showed a consistent increase with later planting dates in 3 years for the three genotypes studied. The low linolenic acid soybean lines were more stable for linolenic acid across planting dates than was Century. Regression coefficients of linolenic acid on mean daily maximum air temperature were - 4.9 g kg^{-1} $°C^{-1}$ for Century and - 3.0 g kg^{-1} $°C^{-1}$ for C1640, in the 0 to 20 days prior to maturity. Temperature during the final 20 days prior to maturity had the highest correlation with linolenic acid content among the different plant reproductive periods studied.

Schnebly and Fehr (1993) conducted a study over 3 years with four planting dates in Iowa. They tested common cultivars and modified fatty-acid genotypes (elevated palmitic, elevated stearic and reduced linolenic acids). They found a significant year effect for all fatty acids, but planting date effects were not as consistent and showed significant interaction with genotypes and years. Reduced linolenic acid genotypes exhibited greater stability than common cultivars among

years. ‘Hardin’ and Century showed a 12 and 13 g kg^{-1} difference in linolenate among years, respectively, as opposed to 3 g kg^{-1} difference for A16 and A17. In contrast, stearic acid content of the elevated stearic acid genotype fluctuated more among years than that of common cultivars. Regression coefficients for each genotype of fatty acid levels on average daily high temperature were variable among genotypes. No significant relationship was found between temperature and fatty acid concentration within most of the modified genotypes studied which differs from other studies. This suggests there are interactions of other factors along with temperature affecting fatty acid levels in the seed oil.

Dornbos and Mullen (1992) evaluated the effect of temperature and drought-stress levels, under controlled conditions, on the fatty acid composition of soybean seed oil. Linoleic and linolenic acid decreased and oleic acid increased with increased temperature during seed fill which agreed with previous studies. They also found slight but inconsistent differences in fatty acid composition under the different drought-stress levels.

Boydak et al. (2002) reported significant differences in oleic and linoleic acid content under four irrigation treatments in 2 years in Turkey. Linoleic acid increased and oleic acid decreased when plants were irrigated every 3rd day after emergence as compared to every 6th, 9th and 12th day. The effect of temperature was not studied.

Flagella et al. (2002) conducted a planting date and irrigation study over 2 years in Italy with two high oleic sunflower hybrids. They found significant effects from irrigation on both years. Oleic and stearic acid decreased while linoleic and

palmitic acids increased under irrigation. Both oleic and linoleic acid contents were significantly affected by planting date. Late planting dates in this study were associated with higher mean temperatures during seed filling, and produced higher oleic acid content and lower linoleic acid content in the seed oil.

Primomo et al. (2002) studied the effect of environment at four locations over 3 years on the fatty acid profile of three soybean cultivars and 14 lines with modified fatty acid composition. The year effect was significant for all fatty acids in their study, while the location effect was significant only for oleic and linolenic acid content. They proposed temperature and precipitation as the factors contributing to these effects. The stability across environments of the fatty acid profile of each genotype was studied by comparing b-values according to Finlay and Wilkinson (1963). Most lines with modified fatty acid levels had more stable fatty acid profiles than normal cultivars except for lines with elevated stearic acid content. Elevated oleic acid genotypes showed differences in stability. Genotype RG9 was more stable while genotype AN145-66 varied more for oleate across environments. AN145-66 has several minor genes responsible for high oleic content, which may be more affected by the environment than the single major gene responsible for higher oleate in RG9 (Primomo et al., 2002).

Bennett et al. (2004) conducted a 2-year study at Stoneville, MS using four soybean cultivars from maturity groups IV and V to evaluate the effect of planting date and irrigation on accumulation of soybean isoflavones. They performed fatty acid analyses of the soybean oil and found only slight differences in the fatty acid profiles. Neither planting date nor irrigation treatment was found to significantly

affect the fatty acid composition of the oil in these cultivars with common fatty acid profiles.

Experimental objectives

New soybean genotypes with modified fatty acid composition are being developed and will be important in meeting industry demands for food, fuel and other uses (Wilson, 2004). It is important to determine best growing conditions for different genotypes with modified oil to insure that fatty acid levels desired by the industry are consistently met. The stability of the fatty acid profile determines the adaptability of each genotype to grow under different temperature and water regimes without significant changes in its oil composition. The presence of genotype x environment interaction for levels of palmitic, stearic, oleic, linoleic and linolenic acids in soybean seed in response to planting dates and temperature in various studies clearly shows the environment affects oil composition. The inconsistency of responses from the few irrigation experiments, however, provides no clear results of the influence of irrigation on the fatty acid composition. Since irrigation coupled with planting dates varying by as much as 2 months is common in many soybean production areas, more research is needed to study the effects of these factors on genotypes with modified fatty acid content in different areas. The objectives of this study were to assess the influence of location, planting date, and irrigation on the fatty acid composition and stability of soybean seed oil in genotypes with modified fatty acid levels.

Chapter II

Materials and Methods

Four soybean cultivars and fifteen soybean genotypes with modified fatty acid levels in the seed oil were used in this study. The cultivars and modified fatty acid genotypes, obtained through induced mutation, recombination, and selection, represent seven fatty acid profiles as follows: 1) reduced linolenic, 2) reduced palmitic, 3) elevated palmitic, 4) mid-oleic, 5) mid-oleic and reduced linolenic, 6) reduced palmitic and reduced linolenic, and 7) common cultivars with typical fatty acid profiles. The genotypes and cultivars used in the study range from maturity group III to V (Table 2.1).

The experiment was conducted in 2004 at five locations (Columbia, Portageville loam soil, Portageville clay soil, Sandhills, NC and Stoneville, MS). Locations, planting dates, soil types and herbicides used are listed in Table 2.2. The experimental design was a split-plot randomized complete block design with two or three blocks at each location. Main plots consisted of irrigated and non-irrigated treatments and subplots consisted of a factorial arrangement of two planting dates and 19 genotypes. Two planting dates were used at each location (early and late) separated by about 30 days (Table 2.2). The two planting dates combined with the 19 genotypes comprised 38 subplots in each main plot. Subplots consisted of three rows, 61 cm long, with 76 cm between rows, except for Stoneville and Raleigh, where row width was 66 cm and 96 cm, respectively. Planting was done by hand with a seeding rate of 60 seed per plot (about 33 seed m^{-1} of row).

Three blocks (replications) were planted at Columbia, Portageville loam soil, Sandhills (NC) and Stoneville (MS) and two blocks were planted on Portageville clay soil. Due to emergence problems the first block at Stoneville had too many missing plots and therefore was not included in the statistical analysis. Also, in the second planting date at Stoneville most of the maturity group V lines had problems with green stems and delayed maturity producing only a few shrunken seeds that were not representative for fatty acid analysis. Therefore, maturity group V data at Stoneville were excluded from all analyses. Two genotypes, S01-9209 and N87-2122-4, at all locations were not included in the analyses. S01-9209 had mixed flower and pubescence color and the fatty acid data were highly variable, likely due to a mixture of two different lines. N87-2122-4 seed had poor germination which resulted in too many missing plots. Therefore the complete data set for analysis consisted of 17 genotypes, 10 of maturity groups III and IV and 7 of maturity group V.

Irrigated plots were sprinkler irrigated at Columbia and Sandhills, and furrow irrigated at Portageville and Stoneville. Irrigation was applied according to the crop needs at each location (Table 2.3).

Data on number of days to first flowering and number of days to maturity (R8) were collected. Table 2.4 shows number of days to maturity at each location for each planting date. The irrigated and non-irrigated treatments produced only slight maturity differences of about 1 day; therefore, data for the two irrigation treatments were pooled together.

The concentrations of palmitic, stearic, oleic, linoleic and linolenic acids as a percentage of the total fatty acids in the seed oil were evaluated for each plot by randomly selecting four plants from the center row and picking two pods each from the seventh and eighth node down from the top of the plant. The 16 pods represented a bulked-plot sample and were later threshed by hand. From each sample 10 seeds were randomly selected for fatty acid analysis. Each 10 seed sample was placed in a paper envelope, manually crushed with a hammer and put in separate test tubes for fatty acid extraction. Crushed tissue was extracted in 5 mL chloroform:hexane:methanol (8:5:2, v/v/v) overnight. Derivitization was done by transferring 100 µL of extract to vial and adding 75 µL of methylating reagent (0.25 *M* methanolic sodium methoxide:petroleum ether:ethyl ether, 1:5:2 v/v/v). Hexane was added to dilute samples to approximately 1 mL. An Agilent (Palo Alto, CA) series 6890 capillary gas chromatograph fitted with a flame ionization detector (275°C) was used with an AT-Silar capillary column (Alltech Associates, Deerfield, IL). Standard fatty acid mixtures (Animal and Vegetable Oil Reference Mixture 6, AOACS) were used as calibration reference standards.

Mean monthly temperature and rainfall during the 2004 growing season at each location are plotted in Figures 2.1 to 2.8 compared to their corresponding 30-year average. In general, rainfall was above average in 2004 at all locations except Sandhills. Stoneville had extreme precipitation peaks with over 30 cm in June and November while having below average rainfall in August and September. All locations had a relatively cool summer with mean temperatures below average levels, which together with good water availability may have

delayed the maturity of soybeans (Figures 2.1 – 2.8). Fall temperatures were above the monthly average and there was no frost that affected the soybean genotypes at any location in this study.

Analysis of variance was conducted using PROC MIXED in SAS for palmitic, stearic, oleic, linoleic and linolenic acid content. Location, irrigation, planting date and genotypes were considered fixed effects while blocks were considered random. Oleic acid content and linolenic acid content showed heterogeneity of variance within each location. Variance increased with increasing levels of each of these fatty acids; therefore, a transformation was performed on these variables to obtain homogeneous variance. A square root transformation provided homogeneous variance for oleic acid content while a natural log transformation provided homogeneous variance for linolenic acid content. Since variance was homogeneous between locations according to Bartlett's test, a pooled location analysis of variance was performed. Mean comparisons were made according to Fisher's LSD at 0.05 significance level.

A phenotypic stability analysis was performed by regression of the fatty acid content of each genotype on an environmental value (Finlay and Wilkinson, 1963). A modified approach of the one used by Finlay and Wilkinson (1963) was used to obtain a quantitative measure of the environment. The average temperature during the 30 days prior to maturity was used as a quantitative measure of the environment instead of the environmental index. Genotypes having stability regression coefficients (b-values of the regression) closer to zero

are more stable while those that deviate significantly from zero (either positive or negative) are considered less stable to changes in the environment.

Table 2.1. Phenotype and corresponding fatty acid profile obtained for each genotype used in the study.

		Fatty Acid Profile*				
Genotype	Phenotype	16:0	18:0	18:1	18:2	18:3
IA 3017	Reduced Linolenic	11.0	4.2	27.6	56.0	1.1
IA 3018	Reduced Linolenic	10.5	3.7	24.2	59.3	2.3
S01-9370	Reduced Linolenic	10.4	3.7	20.9	61.3	3.7
C1943	Reduced Palmitic	4.0	3.7	34.3	51.3	6.8
S01-9267**	Reduced Palmitic	4.4	3.0	26.5	58.8	7.4
N 87-2122-4	Reduced Palmitic	-	-	-	-	-
C1727	Elevated Palmitic	15.2	3.6	19.5	53.6	8.1
M23**	Mid Oleic	9.4	3.6	44.9	35.4	6.6
N 97-3363-4	Mid Oleic & Reduced Linolenic	8.5	4.1	49.8	34.8	2.7
N 98-4445A	Mid Oleic & Reduced Linolenic	9.1	4.2	53.3	30.7	2.7
Holl**	Mid Oleic & Reduced Linolenic	9.2	3.6	43.5	39.7	4.0
CR03-529**	Mid Oleic & Reduced Linolenic	10.9	4.5	38.3	42.8	3.5
MD 00-6605	Reduced Palmitic & Reduced Linolenic	4.1	3.9	30.8	57.5	3.6
MD 99-5458**	Reduced Palmitic & Reduced Linolenic	4.0	3.9	25.2	62.8	4.1
S01-9209	Reduced Palmitic & Reduced Linolenic	-	-	-	-	-
DKB 38-52	Regular Cultivar	11.2	3.7	23.5	54.9	6.7
MPV 457	Regular Cultivar	10.2	4.4	25.5	52.7	7.2
AG 4902**	Regular Cultivar	10.4	4.1	23.7	54.1	7.7
Manokin**	Regular Cultivar	11.3	4.4	21.3	55.6	7.3

* average of five locations and two planting dates except for genotypes marked ** which are average of four locations and two planting dates

Table 2.2. Planting dates, soil types and herbicides applied at each of five locations, 2004.

Location	Planting dates	Soil type	Herbicides
Columbia, MO (Bradford Res. & Extension Center)	May 12, 2004 June 14, 2004	Mexico silt loam	S-metolachlor Lactofen Imazaquin Chloransulam
Portageville, MO Loam soil (Lee Farm)	April 29, 2004 June 2, 2004	Tiptonville silt loam	Fomesafen Clethodim
Portageville, MO Clay soil (Lee Farm)	April 29, 2004 June 2, 2004	Portageville clay	Fomesafen Clethodim
Sandhills, NC (Sandhills Res. Station)	May 26, 2004 June 25, 2004	Candor Sand	S-metolachlor Sulfentrazone Chlorimuron etil Basagran Chloransulam-methyl
Stoneville, MS (USDA Res. Exp. Station)	April 28, 2004 June 8, 2004	Tunica silty clay	S-metolachlor Fomesafen Clethodim Imazaquin

Table 2.3. Dates and amounts of irrigation applied at each of five locations, 2004.

Columbia		**Port. Loam**		**Port. Clay**		**Sandhills**		**Stoneville**	
Date	cm	Date	cm	Date	cm	Date	cm	Date	cm
7/21	**1.5**	**6/10**	**5.1**	**6/11**	**5.1**	**7/7**	**2.5**	**8/3**	**2.5**
8/3	**1.0**	**7/15**	**5.1**	**7/14**	**5.1**	**7/23**	**1.5**	**8/13**	**2.5**
8/13	**2.5**	**8/13**	**5.1**	**8/13**	**5.1**	**7/29**	**2.5**	**9/9**	**2.5**
9/10	**1.5**					**8/12**	**2.5**		
9/24	**2.5**					**8/20**	**2.5**		
						8/27	**1.9**		
Total	**9.0**		**15.3**		**15.3**		**13.6**		**7.5**

Table 2.4. Number of days to maturity (R8) at each of five locations and two planting dates, 2004.

	Columbia		Portageville Loam		Portageville Clay		Sandhills, NC		Stoneville, MS		Average
	Date 1	Date 2	Date 1	Date 2	Date 1	Date 2	Date 1	Date 2	Date 1	Date 2	
Genotypes											
C 1727	122	107	105	102	109	104	96	93	89	90	105
IA 3017	130	109	113	103	116	103	100	95	102	93	109
IA 3018	133	112	116	107	117	108	100	94	100	94	111
DKB 38-52	137	114	127	108	133	111	106	100	106	97	117
C 1943	135	112	136	107	139	110	111	98	110	100	118
N 98-4445A	137	121	135	108	138	112	112	99	110	98	120
N 97-3363-4	139	123	136	111	137	112	109	99	110	104	120
MPV 457	142	119	136	112	139	117	119	102	110	105	123
MD 00-6605	144	123	137	112	140	116	120	102	116	108	124
S01-9370	147	127	135	114	136	118	118	103	114	105	125
AG 4902	146	124	137	118	141	118	120	107	125	109	126
CR 03-529	149	133	138	113	144	117	120	107	120	109	128
MD 99-5458	158	136	144	122	151	122	130	113	143	-	134
MANOKIN	156	135	146	123	155	124	129	112	143	-	135
M 23	159	141	144	123	151	122	131	111	155	-	135
Holl	161	140	147	123	151	126	129	113	150	-	136
S01-9267	166	143	147	124	153	123	124	110	148	-	136
Average											
all lines	144	125	134	114	138	115	116	103	121	-	124

Figure 2.1. Mean monthly temperature, April – November, 2004 and 30-year average, Columbia, MO.

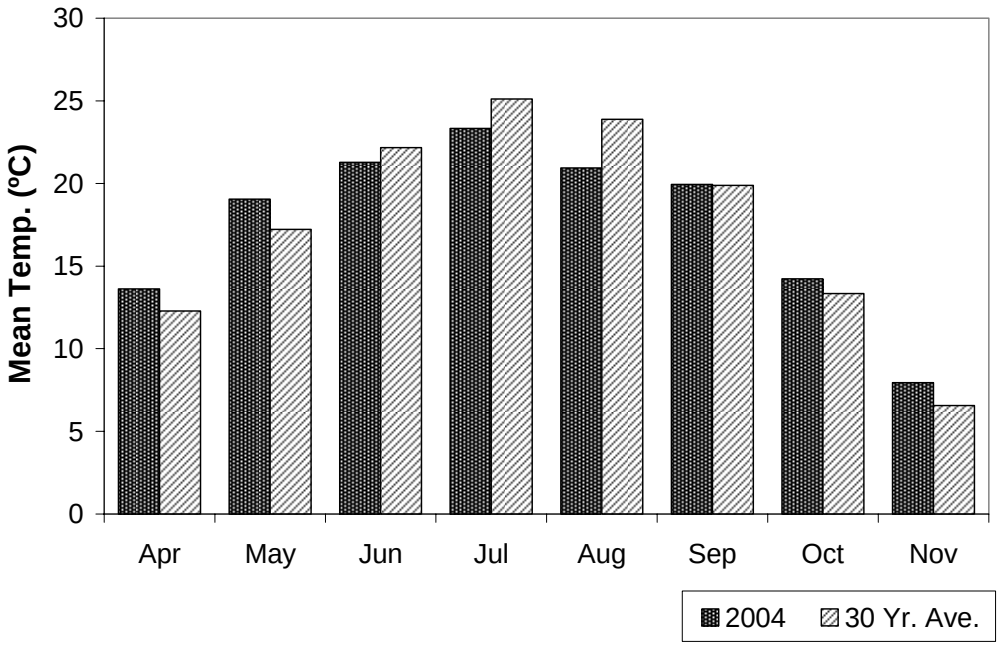


Figure 2.2. Monthly rainfall, April – November, 2004 and 30-year average, Columbia, MO.

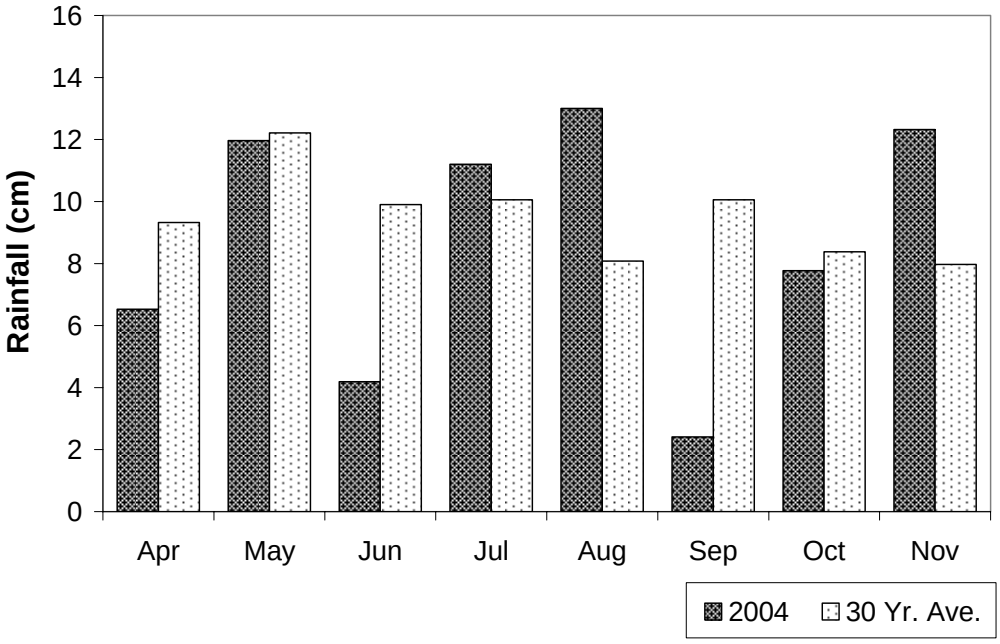


Figure 2.3. Mean monthly temperature, April – November, 2004 and 30-year average, Portageville, MO.

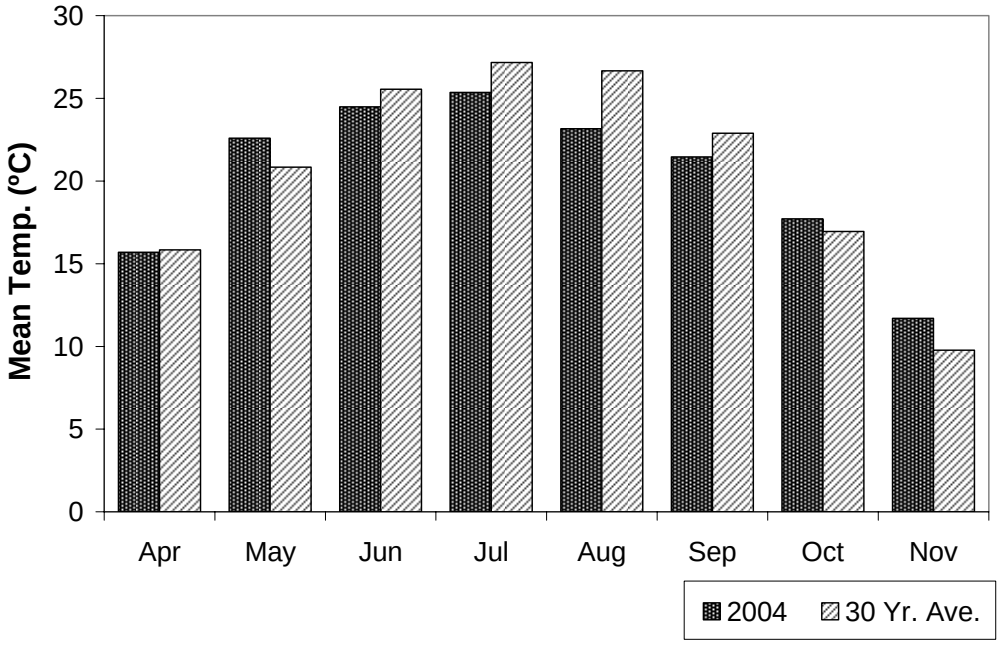


Figure 2.4. Monthly rainfall, April – November, 2004 and 30-year average, Portageville, MO.

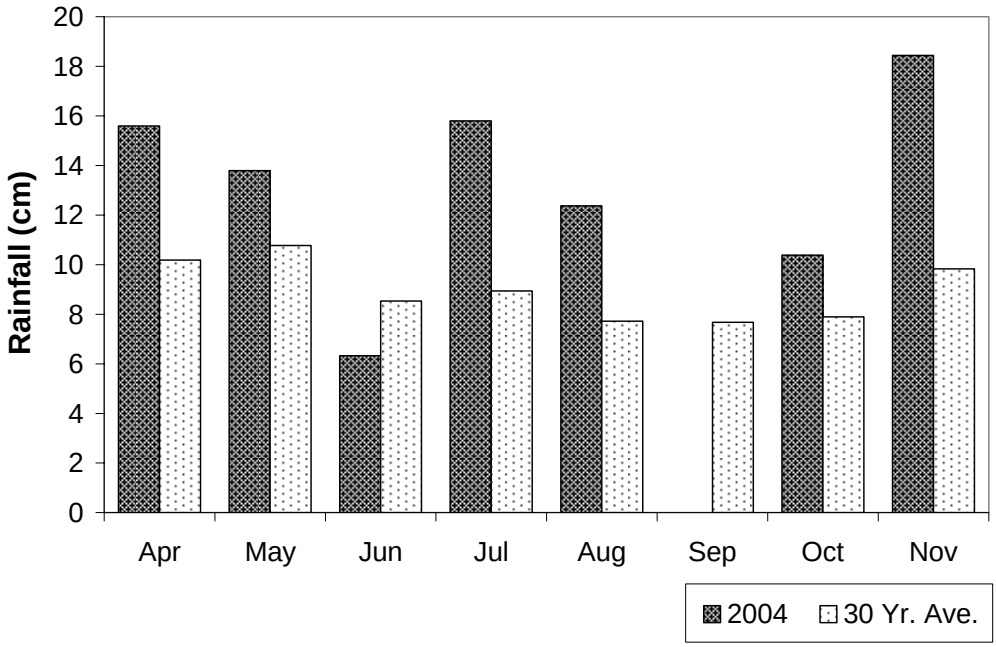


Figure 2.5. Mean monthly temperature, April – November, 2004 and 30-year average, Sandhills, NC.

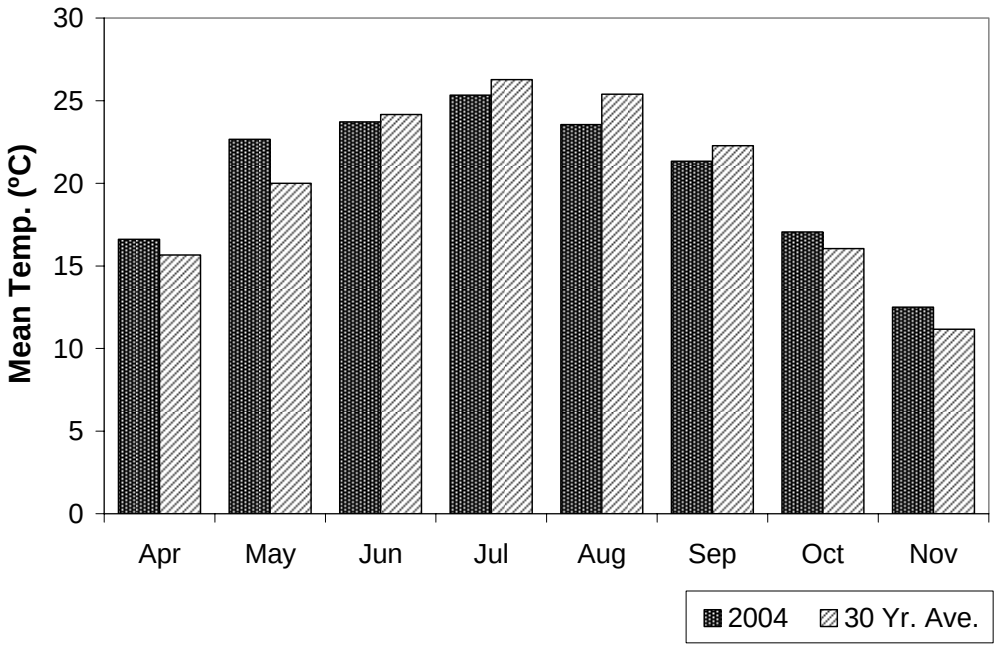


Figure 2.6. Monthly rainfall, April – November, 2004 and 30-year average, Sandhills, NC.

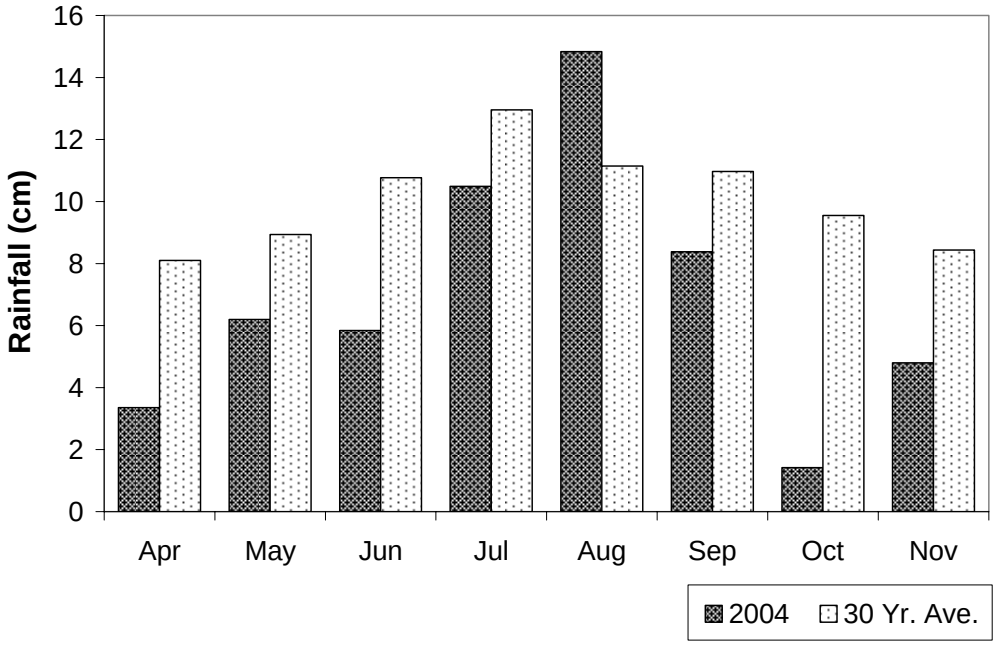


Figure 2.7. Mean monthly temperature, April – November, 2004 and 30-year average, Stoneville, MS.

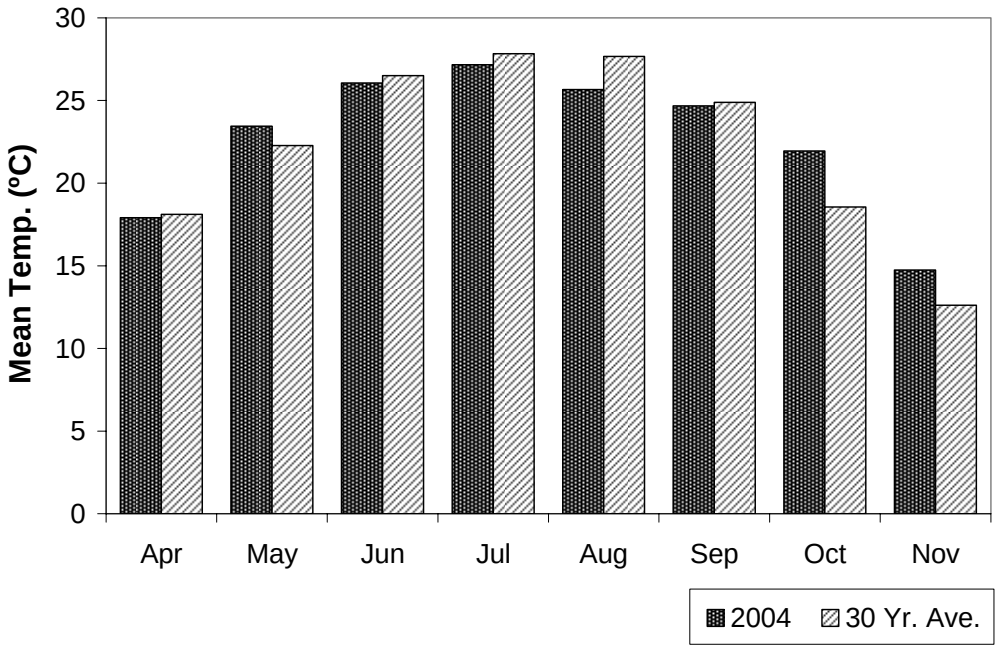
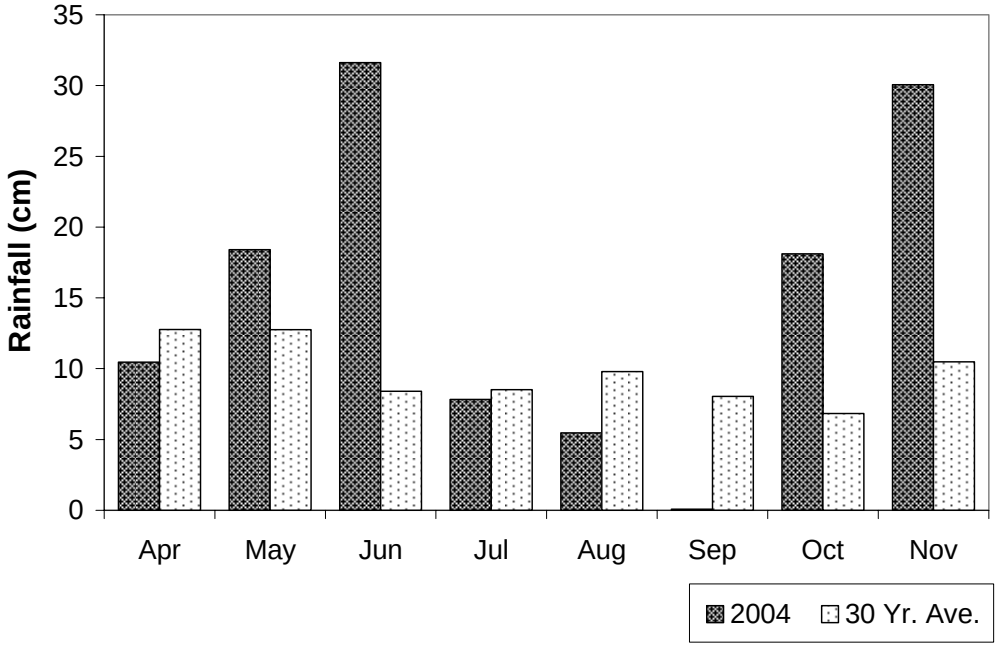


Figure 2.8. Monthly rainfall, April – November, 2004 and 30-year average, Stoneville, MS.



Chapter III

Analysis of Variance

Analysis of variance was performed to test the effects of location, planting date, irrigation, and their interactions on the levels of five fatty acids in soybean seed oil of 17 genotypes with varying fatty acid profiles. Fisher's LSD at the 0.05 significance level was used to test for significant differences among treatments.

All maturity group III, IV and V genotypes produced normal seeds at all five locations and each of the two planting dates. In the second planting date at Stoneville, however, most maturity group V genotypes produced very small seeds that were not reliable for fatty acid analysis. Therefore, analysis was performed separately for the early maturity genotypes (MG III and IV) and late maturity genotypes (MG V). Five locations (Columbia, Portageville Loam Soil, Portageville Clay Soil, Sandhills and Stoneville) were used in the analysis of maturity groups III and IV. Four locations were used in the maturity group V analysis, since data from Stoneville were not included.

Maturity Group III and IV

To properly perform the analysis of variance the assumptions of normality and homogeneity of variance were tested. Oleic and linolenic acid content showed heterogeneous variance. Variance increased with increasing levels of each of these fatty acids. Therefore, data were transformed to obtain homogeneous variance. A square root transformation was needed for oleic acid

content and a natural log transformation was needed for linolenic acid content to provide homogeneity of variance. Figures 3.1 and 3.2 show the residual plots of the transformed variables oleic and linolenic acid contents, respectively. Palmitic, stearic and linoleic acid contents were normally distributed and showed homogeneous variance.

Error terms for within location effects were compared between the five locations with Bartlett's test. The five fatty acids showed homogeneous error terms across locations. Therefore, a pooled location analysis of variance was performed for the five fatty acids and is shown in Table 3.1.

Palmitic acid content

Palmitate content showed a significant irrigation x planting date interaction. It significantly increased under irrigation in the early planting date and increased slightly in the late planting date, although not statistically significant at $p<0.05$ (Figure 3.3). This effect was consistent across locations as shown by the non-significant location x irrigation and location x irrigation x planting date interactions (Table 3.1). Similar increases in palmitate content under irrigation were found in sunflower (Flagella et al., 2002).

Water status in the plant may have effects on the relative activity of the enzymes in the fatty acid elongation and desaturation pathways, and therefore affect the levels of palmitic acid in the seed. Interactions of the water status in the plant with temperature may also importantly affect the palmitic acid content,

suggesting the variability among planting dates and locations may be caused by the interaction of temperature with rainfall at each of the environments.

Genotypes showed different responses to planting dates and locations for palmitate content (Table 3.2). At Columbia and Sandhills there was a small effect of planting date but this effect varied between positive and negative values among different genotypes. At both Portageville locations palmitic content decreased in the late planting date in most genotypes whereas at Stoneville it increased in the late planting date on most genotypes. The inconsistent planting date effects among locations and genotypes along with slight differences among means at different locations, shows that temperature has little consistent influence on palmitic acid content. Factors other than temperature appear to affect variability of palmitate in various environments. Other studies showed similar results with regard to temperature effects on palmitic acid content (Wolf et al., 1982; Dornbos and Mullen, 1992; Primomo et al., 2002).

Stearic acid content

Stearic acid content decreased with higher water availability due to irrigation (Table 3.1). This effect was consistent across locations, planting dates, and genotypes. The irrigation effect was significant in both the early and late planting dates (Figure 3.4). Dornbos and Mullen (1992) reported increases in the proportion of stearic acid in seed from ‘Hodgson 78’ under drought stress treatments. Our results also showed a decrease in stearic acid content with higher water availability.

Stearic acid content showed a significant location x planting date interaction and genotype x location x planting date interaction. Table 3.3 shows the mean stearic acid content for each genotype with corresponding LSD values at each location and planting date. An increase in stearate at the late planting dates was consistent across genotypes for Columbia and Stoneville. At both Portageville locations stearate content increased except for the high oleic genotypes (N 97-3363-4 and N 98-4445A) in which stearate decreased in the late planting. This may be due to specific factors that affected these two lines differently than the rest of the genotypes. The Sandhills location showed an opposite response in which stearic acid content decreased for all genotypes with late planting.

With the exception of Sandhills, the other locations showed that higher temperatures were associated with a decrease in stearic acid content. Even though Sandhills location did not have the highest temperatures, mean stearate contents of genotypes were the lowest compared to the other locations. This may be due to the combination of relatively high temperatures and other weather related factors affecting stearic acid content at that location. Previous studies were not consistent on the effect of temperature on stearic acid content. Wolf et al. (1982) reported no effect of temperature on stearic acid content. Dornbos and Mullen (1992) reported a significant increase in stearic acid content with increases in temperature for Hodgson 78. Rennie and Tanner (1989) reported increases in stearic acid content when soybean lines were grown at 28/22ºC

(day/night) compared to 15/12ºC and decreases in stearic acid content when grown at 40/30ºC as compared to 28/22ºC.

Unsaturated fatty acids, oleate, linoleate, and linolenate

All three unsaturated fatty acids showed a significant irrigation x location x planting date interaction. Therefore, the irrigation effect on unsaturated fatty acid content was not consistent across locations and planting dates. Irrigated treatments generally showed lower oleic acid content or no difference, except for the Sandhills location where the irrigated treatment had higher oleic acid content (Figure 3.5). Linolenic acid content among genotypes either decreased or remained similar in non-irrigated treatments except for the second planting date at the Sandhills location where linolenic acid content increased slightly (Figure 3.6). Although interactions were present, in general these results from irrigation are in agreement with those of Boydak et al. (2002) in soybean and Flagella et al. (2002) in sunflower. Since location and planting date combinations provide variable environments, different weather patterns for temperature and rainfall distribution may be the underlying factors for this irrigation x location x planting date interaction.

A significant genotype x location x planting date interaction is present for the three unsaturated fatty acids. From a pooled analysis of the genotypes, it is clear that a different response was shown for the three unsaturated fatty acids to planting dates at Portageville Loam and Portageville Clay as compared to Columbia, Sandhills and Stoneville locations (Figures 3.7, 3.8 and 3.9). Analysis

of weather variables like average temperature during the seed filling period at each location and planting date combination provides some insight on factors underlying this interaction. The average temperature at the Portageville locations tended to be similar for the early and late planting dates. Therefore, the variation in the unsaturated fatty acid contents among planting dates may be due to rainfall. Since September was very dry at Portageville the late planting date matured in a drier environment and that may be partially responsible for the higher oleic acid content and lower linolenic acid content. At the other locations (Columbia, Sandhills, and Stoneville) there was a decrease in the average temperature for the second planting date which is consistent with the lower oleate and higher linolenate levels at late planting dates.

The location mean temperatures also showed consistency with the unsaturated fatty acid contents across the different locations. These results are in agreement with previous studies on the effect of temperature, and its relation to planting dates and locations, on the levels of unsaturated fatty acids in the seed oil (Howell and Collins, 1957; Cherry et al., 1985; Wilcox and Cavins, 1992; Primomo et al., 2002). The inconsistent effect of planting dates on the levels of unsaturated fatty acids found by Schnebly and Fehr (1993) may be due to particular weather conditions at that location or interactions of temperature during seed filling with other factors like rainfall.

Variability in the mean content of oleate, linoleate and linolenate among locations and planting dates for each genotype is evident in Tables 3.4, 3.5 and 3.6, respectively. Most genotypes reacted consistently with a few exceptions for

response of unsaturated fatty acids to planting date at each location. The magnitude of the responses to planting date at each location differed among genotypes. A stability analysis to measure stability of oleic, linoleic and linolenic acids across environments for each genotype is presented later (Chapter 4) to provide insight on the genotype x environment interaction present in this study. Average temperature during the last 30 days of the seed filling period provided a quantitative measure of the environment (combination of location and planting date) for the stability analysis.

Figure 3.1. Residual plot of square root transformation on oleic acid content for ten maturity group III and IV genotypes, two planting dates, two irrigation levels and five locations, 2004.

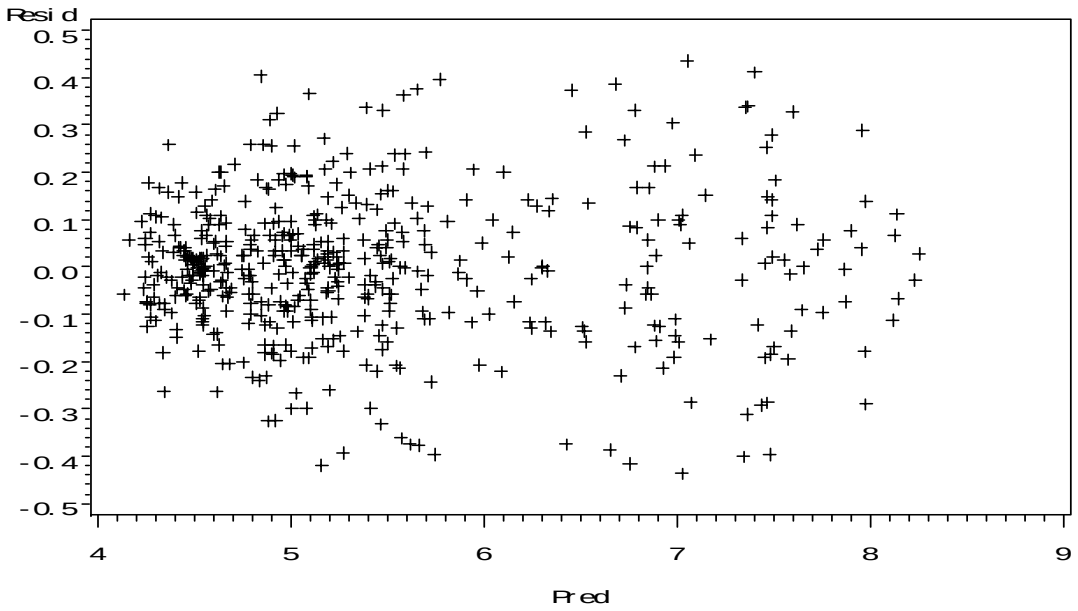


Figure 3.2. Residual plot of natural log transformation on linolenic acid content for ten maturity group III and IV genotypes, two planting dates, two irrigation levels and five locations, 2004.

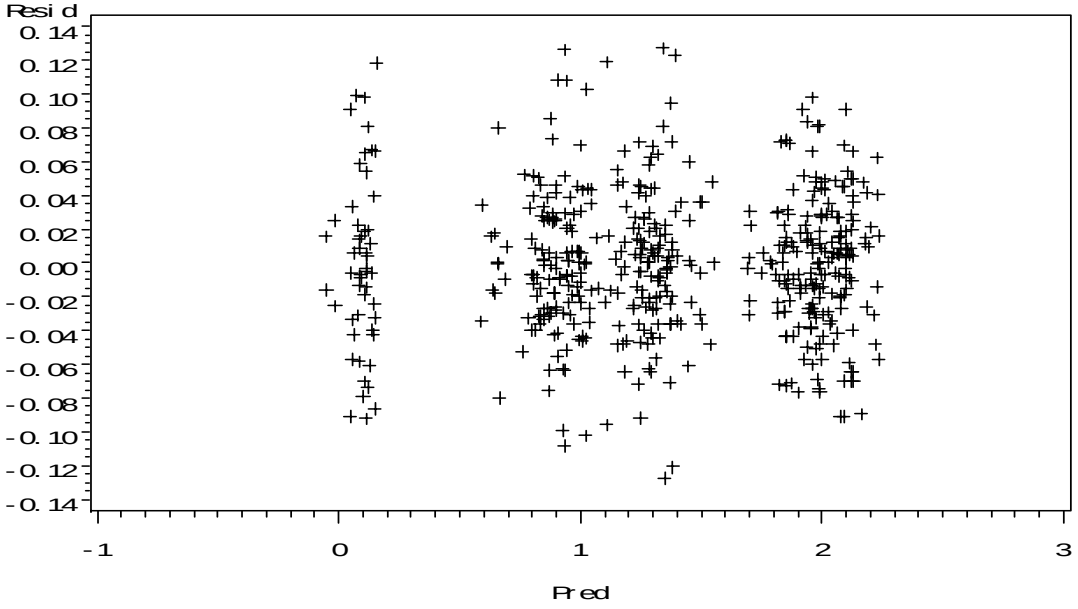
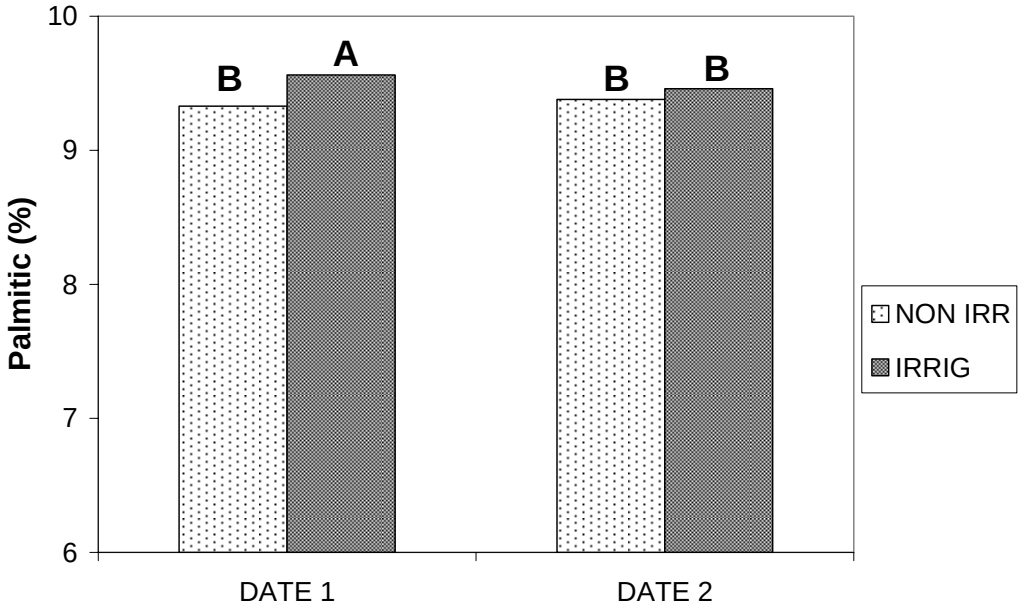


Table 3.1. Pooled analysis of variance across five locations, two irrigation treatments, two planting dates and ten maturity group III and IV genotypes for palmitic, stearic, oleic, linoleic, and linolenic acids, 2004.

		Palmitic	Stearic	Oleic	Linoleic	Linolenic
Source	**df**	**F value**	**F value**	**F value**	**F value**	**F value**
Location (Loc)	4	9.45 **	101.99 **	80.18 **	58.4 **	205.09 **
Irrigation (Irr)	1	4.68 NS	40.17 **	1.4 NS	0.79 NS	7.71 *
Loc x Irr	4	2.76 NS	0.45 NS	3.48 NS	3.29 NS	1.33 NS
Planting Date (Date)	1	0.48 NS	25.01 **	11.9 **	13.05 **	41.56 **
Loc x Date	4	25.32 **	56.4 **	97.89 **	78.47 **	58.65 **
Irr x Date	1	4.09 *	3.36 NS	0.3 NS	2.64 NS	0.18 NS
Loc x Irr x Date	4	1.33 NS	1.99 NS	3.42 **	3.1 *	3.31 *
Genotype (G)	9	3041.56 **	63.92 **	1212.53 **	1139.67 **	6541.52 **
G x Loc	36	3.68 **	15.48 **	18.03 **	20.53 **	12.42 **
G x Irr	9	1.51 NS	1.05 NS	0.64 NS	0.86 NS	1.17 NS
G x Loc x Irr	36	1.63 *	0.78 NS	1.71 **	1.87 **	0.62 NS
G x Date	9	1.34 NS	8.67 **	9.15 **	9.64 **	4.34 **
G x Loc x Date	36	1.97 **	3.58 **	6.97 **	7.91 **	5.59 **
G x Irr x Date	9	0.75 NS	0.79 NS	1.42 NS	1.5 NS	1.16 NS
G x Loc x Irr x Date	36	1.28 NS	1.29 NS	1.28 NS	1.38 NS	1.27 NS
Pooled Error	292					

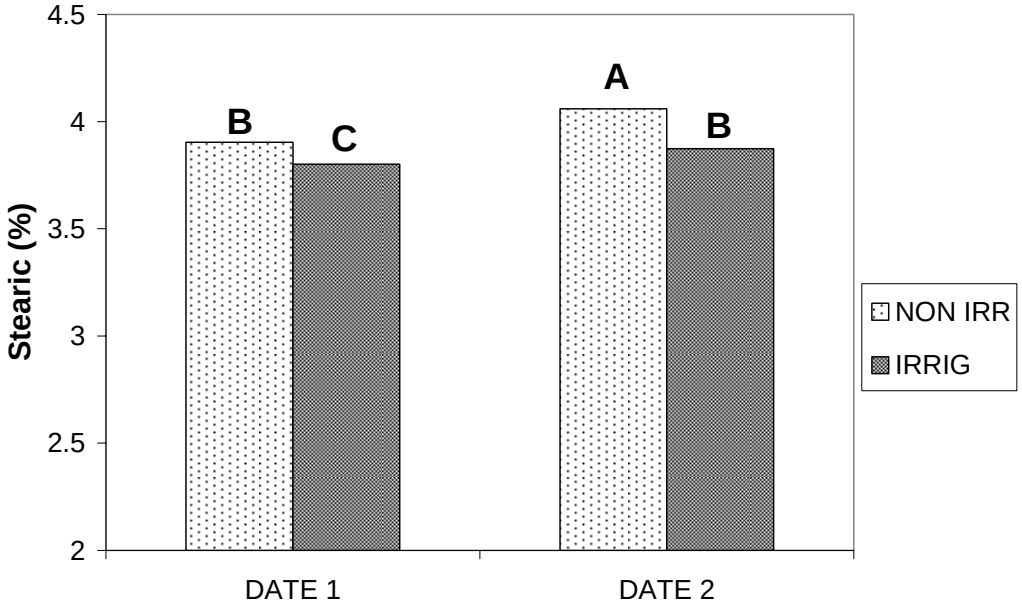
*, ** Significant at the 0.05 and 0.01 probability levels, respectively. NS = not significant.

Figure 3.3. Effect of irrigation on mean palmitic acid content (%) averaged across ten maturity group III and IV genotypes and five locations at each of two planting dates, 2004.



* Means with the same letter do not differ at the 0.05 probability level.

Figure 3.4. Effect of irrigation on mean stearic acid content (%) averaged across ten maturity group III and IV genotypes and five locations at each of two planting dates, 2004.



* Means with the same letter do not differ at the 0.05 probability level.

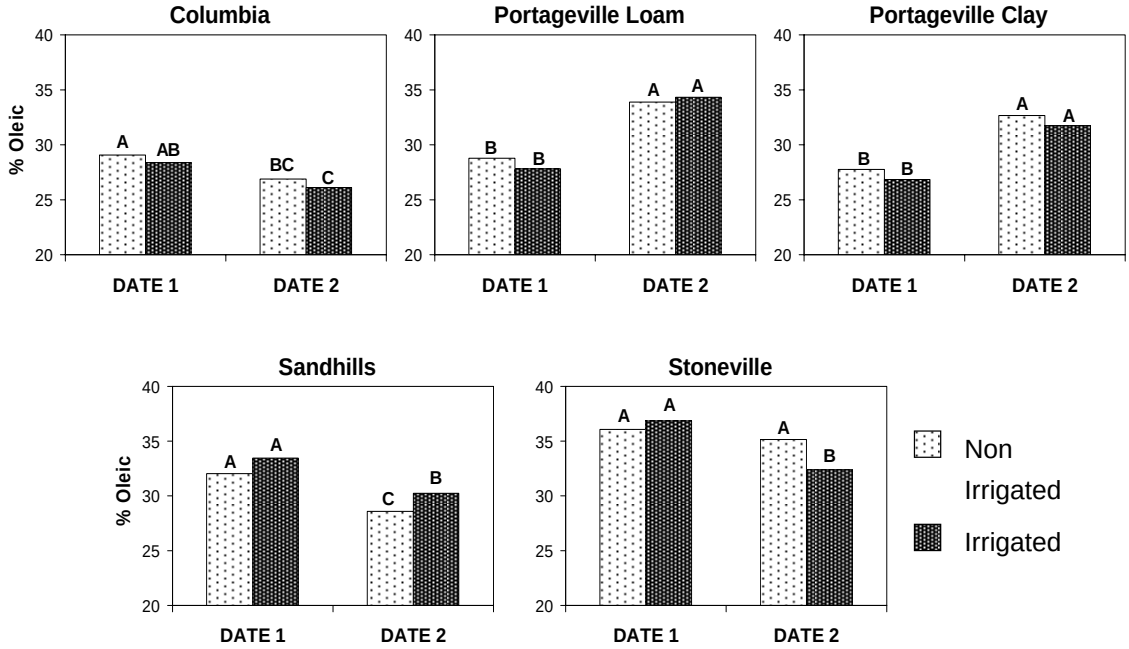
Table 3.2. Mean palmitic acid content (%) for ten maturity group III and IV genotypes for two planting dates at each of five locations, 2004.

	Columbia		Port. Loam		Port. Clay		Sandhills		Stoneville		
Genotypes	Date 1	Date 2	Date 1	Date 2	Date 1	Date 2	Date 1	Date 2	Date 1	Date 2	LSD
IA 3017	10.7	10.6	11.3	10.5	11.1	10.7	11.4	11.3	10.9	11.5	0.6
IA 3018	10.1	10.2	10.5	10.2	10.7	10.5	10.8	10.9	10.5	10.8	0.6
S01-9370	9.9	9.7	10.3	10.0	10.5	10.2	11.0	10.6	10.5	11.0	0.5
C 1943	3.8	4.2	4.1	4.2	4.0	3.8	3.9	4.2	3.5	3.9	0.8
C 1727	14.9	14.8	15.2	14.3	15.3	15.0	15.8	15.7	15.2	16.2	1.0
N 97-3363-4	8.6	8.8	8.5	7.9	9.0	8.8	8.3	8.6	8.0	8.7	0.8
N 98-4445A	9.0	9.0	9.2	8.2	10.2	8.6	9.4	9.3	8.2	10.2	0.8
MD 00-6605	4.3	3.9	3.9	3.7	4.3	4.1	4.1	3.9	3.8	4.7	0.5
DKB 38-52	10.7	11.0	11.0	10.7	11.2	11.3	11.7	11.8	11.2	12.0	0.8
MPV 457	9.6	9.6	10.1	10.1	10.3	10.0	11.0	10.6	10.6	10.5	0.4

Table 3.3. Mean stearic acid content (%) for ten maturity group III and IV genotypes for two planting dates at each of five locations, 2004.

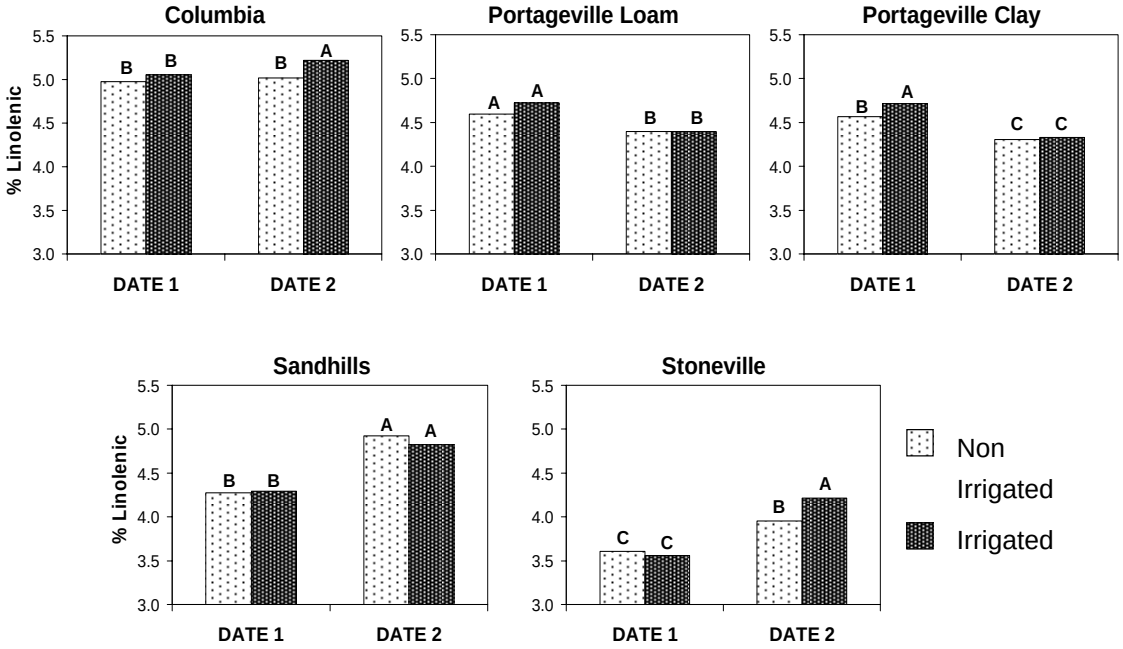
	Columbia		Port. Loam		Port. Clay		Sandhills		Stoneville		
Genotypes	Date 1	Date 2	Date 1	Date 2	Date 1	Date 2	Date 1	Date 2	Date 1	Date 2	LSD
IA 3017	4.5	5.2	4.1	4.4	4.2	4.3	4.1	3.6	3.6	4.4	0.4
IA 3018	3.9	4.5	3.6	3.7	3.8	3.6	3.5	3.3	3.2	3.6	0.3
S01-9370	3.7	3.9	3.8	3.8	3.7	3.8	3.4	3.4	3.5	3.9	0.3
C 1943	4.7	4.8	3.6	3.8	3.7	3.5	3.1	2.9	2.9	3.6	0.4
C 1727	3.8	4.4	3.4	4.1	3.4	3.7	3.3	3.1	3.1	3.4	0.3
N 97-3363-4	5.3	5.6	4.9	3.8	4.7	4.3	3.1	2.7	3.2	3.5	0.5
N 98-4445A	4.8	5.2	4.7	4.2	5.1	4.0	3.5	3.0	3.3	3.8	0.4
MD 00-6605	3.8	4.5	3.9	4.2	3.6	4.0	3.7	3.6	3.8	4.0	0.5
DKB 38-52	3.9	4.4	3.8	3.6	3.7	3.5	3.5	3.2	3.3	4.0	0.4
MPV 457	5.2	5.8	4.4	4.5	4.3	4.4	3.9	3.2	3.5	4.4	0.4

Figure 3.5. Mean oleic acid content (%) averaged across ten maturity group III and IV genotypes with and without irrigation for two planting dates at each of five locations, 2004.



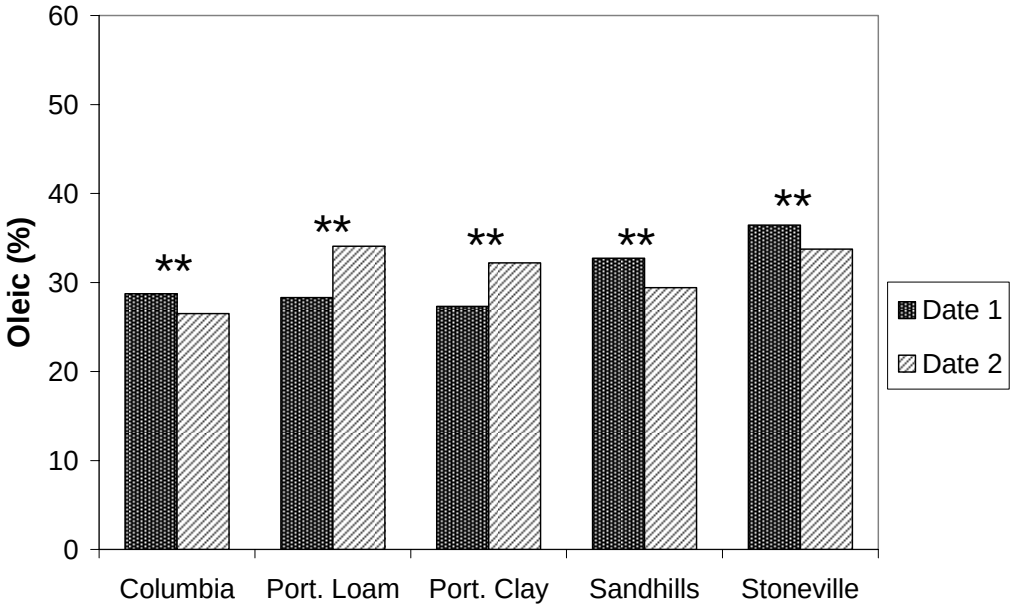
* Means with the same letter at each location do not differ at the 0.05 probability level.

Figure 3.6. Mean linolenic acid content (%) averaged across ten maturity group III and IV genotypes with and without irrigation for two planting dates at each of five locations, 2004.



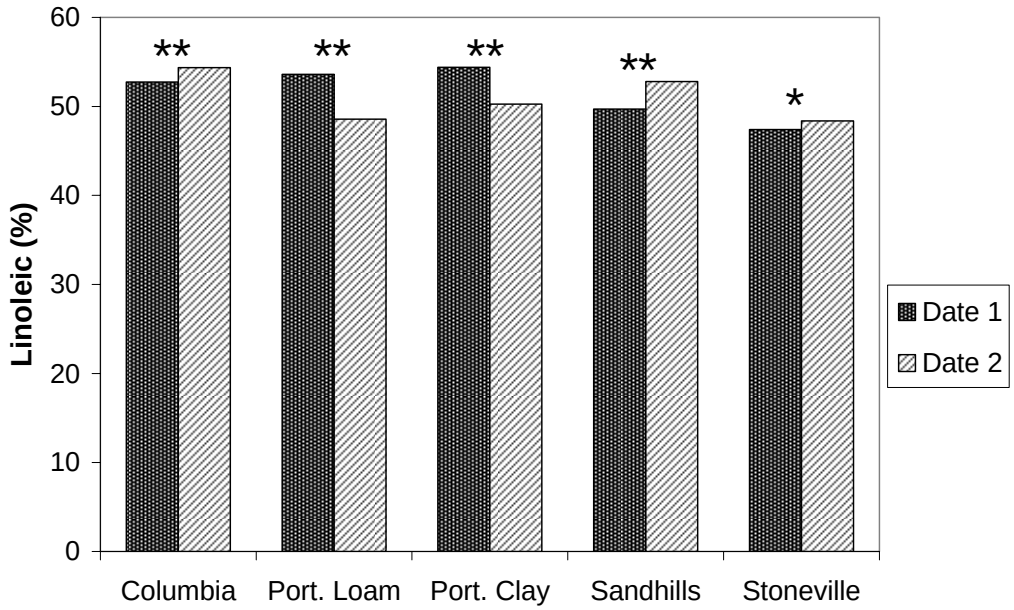
* Means with the same letter at each location do not differ at the 0.05 probability level.

Figure 3.7. Mean oleic acid content (%) averaged across ten maturity group III and IV genotypes for two planting dates at each of five locations, 2004.



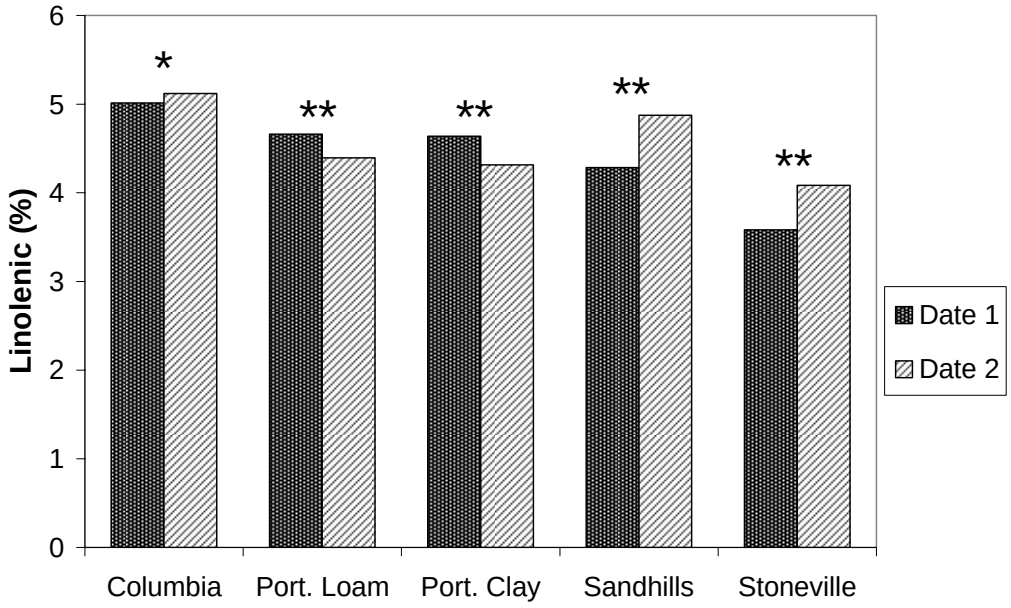
*, ** Significant differences between planting dates at the 0.05 and 0.01 probability levels, respectively.

Figure 3.8. Mean linoleic acid content (%) averaged across ten maturity group III and IV genotypes for two planting dates at each of five locations, 2004.



*, ** Significant differences between planting dates at the 0.05 and 0.01 probability levels, respectively.

Figure 3.9. Mean linolenic acid content (%) averaged over ten maturity group III and IV genotypes for two planting dates at each of five locations, 2004.



*, ** Significant differences between planting dates at the 0.05 and 0.01 probability levels, respectively.

Table 3.4. Mean oleic acid content (%) for ten maturity group III and IV genotypes for two planting dates at each of five locations, 2004.

	Columbia		Port. Loam		Port. Clay		Sandhills		Stoneville		
Genotypes	Date 1	Date 2	Date 1	Date 2	Date 1	Date 2	Date 1	Date 2	Date 1	Date 2	LSD
IA 3017	27.1	26.0	23.2	32.4	24.7	29.4	26.2	24.6	32.5	29.8	3.5
IA 3018	23.6	24.9	20.7	27.4	20.8	26.1	23.3	22.4	28.2	24.6	2.2
S01-9370	20.7	20.6	20.1	21.6	20.2	21.8	20.0	18.7	20.6	24.6	0.9
C 1943	30.0	26.6	29.6	33.0	29.6	36.7	41.3	29.0	44.1	43.0	4.6
C 1727	18.7	20.3	18.6	22.2	17.6	20.0	19.3	18.6	21.4	18.3	2.0
N 97-3363-4	41.1	31.2	48.1	55.1	44.3	48.6	59.7	51.5	64.8	54.0	6.1
N 98-4445A	47.5	39.6	48.1	61.1	45.4	58.0	56.0	56.4	67.1	53.8	5.0
MD 00-6605	26.0	26.5	32.6	31.8	29.0	30.1	32.6	28.3	37.9	33.4	3.1
DKB 38-52	24.1	23.9	19.3	28.8	19.7	23.9	23.0	21.8	24.6	25.7	2.5
MPV 457	28.4	25.6	22.7	27.4	21.8	27.3	26.1	22.8	23.1	30.1	2.7

Table 3.5. Mean linoleic acid content (%) for ten maturity group III and IV genotypes for two planting dates at each of five locations, 2004.

	Columbia		Port. Loam		Port. Clay		Sandhills		Stoneville		
Genotypes	Date 1	Date 2	Date 1	Date 2	Date 1	Date 2	Date 1	Date 2	Date 1	Date 2	LSD
IA 3017	56.6	57.1	60.2	51.6	58.9	54.4	57.2	59.4	52.0	53.2	3.5
IA 3018	59.6	57.9	62.8	56.4	62.4	57.6	60.1	61.0	56.2	58.5	2.1
S01-9370	61.8	61.3	62.2	60.9	61.8	60.5	62.1	63.3	62.0	57.1	1.1
C 1943	53.6	56.1	55.5	52.1	55.8	49.6	45.1	55.7	44.8	44.3	4.0
C 1727	53.2	51.9	54.8	51.9	55.5	53.5	53.8	53.8	53.9	54.1	1.6
N 97-3363-4	41.8	50.6	35.6	30.5	39.3	35.6	26.5	34.6	22.0	31.4	5.4
N 98-4445A	35.5	42.8	35.1	23.9	36.6	26.7	28.6	28.8	19.5	29.9	4.3
MD 00-6605	61.9	61.0	56.1	56.6	59.6	58.1	56.0	60.4	51.7	53.8	2.9
DKB 38-52	54.0	53.5	58.4	50.7	58.1	54.9	55.4	56.1	55.3	52.3	2.1
MPV 457	49.4	51.3	55.2	51.0	55.8	51.6	52.2	54.8	56.4	49.0	2.5

Table 3.6. Mean linolenic acid content (%) for ten maturity group III and IV genotypes for two planting dates at each of five locations, 2004.

	Columbia		Port. Loam		Port. Clay		Sandhills		Stoneville		
Genotypes	Date 1	Date 2	Date 1	Date 2	Date 1	Date 2	Date 1	Date 2	Date 1	Date 2	LSD
IA 3017	1.2	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.0	1.1	0.1
IA 3018	2.7	2.5	2.3	2.3	2.3	2.2	2.3	2.3	1.9	2.4	0.1
S01-9370	3.9	4.4	3.7	3.7	3.7	3.6	3.4	3.9	3.5	3.4	0.2
C 1943	7.9	8.4	7.1	6.9	6.9	6.4	6.6	8.2	4.6	5.1	0.7
C 1727	9.3	8.6	8.0	7.7	8.2	7.8	7.8	8.7	6.4	8.0	0.6
N 97-3363-4	3.2	3.8	2.8	2.6	2.8	2.6	2.4	2.6	2.0	2.5	0.2
N 98-4445A	3.2	3.4	2.9	2.7	2.8	2.6	2.5	2.4	1.9	2.3	0.3
MD 00-6605	4.0	4.1	3.5	3.6	3.6	3.6	3.5	3.8	2.8	4.0	0.4
DKB 38-52	7.2	7.2	7.5	6.3	7.3	6.5	6.4	7.1	5.5	6.0	0.5
MPV 457	7.5	7.7	7.7	7.0	7.7	6.7	6.9	8.5	6.4	6.1	0.5

Maturity group V

Data for seven maturity group V genotypes differing in fatty acid profile (Table 2.1) was used in the analysis of variance over four locations instead of the five used for maturity groups III and IV. Data from Stoneville were not used due to missing plots from germination and problems from stink bug injury in the second planting date that delayed maturity producing small seeds that were not representative of each genotype. Two lines were not used in the analyses, S01-9209 and N87-2122-4. S01-9209 had mixed flower and pubescence color and appeared to be a mixture of two different lines. N87-2122-4 had low germination which resulted in poor stands.

Transformations were performed to obtain homogeneous variance on data for oleic and linolenic acid content as in the maturity group IV analysis. A square root transformation was used for oleic acid content and a natural log transformation was used for linolenic acid content.

Within location error terms were compared among the four locations by Bartlett's test. Error terms were homogeneous for each of the five fatty acids across locations. Therefore, a pooled analysis of variance was performed over the four locations and results are presented in Table 3.7.

Palmitic acid content

Irrigation had no significant effect on palmitic acid content (Figure 3.10). The irrigation x genotype x location interaction was significant as well as the irrigation x genotype x planting date interaction (Table 3.7). Therefore, responses

to irrigation varied among genotypes, locations and planting dates, but did not show any consistent effect. This contrasts with the significant increase in palmitic acid content under irrigation found in the analysis of maturity groups III and IV and with results in sunflower reported by Flagella et al. (2002). Maturity group V genotypes matured later, under different weather conditions than earlier maturity groups. Differences in rainfall and temperature may be the underlying factors to the different results obtained on different maturity groups.

The planting date effect on palmitate content was significant, and also showed significant interactions with locations and genotypes. This indicates that palmitic acid content of genotypes responded differently at the two planting dates at different locations. Mean palmitic acid contents for the seven maturity group V genotypes at two planting dates at each of four locations with corresponding LSD values are shown in Table 3.8. The response to planting dates varied among genotypes and locations. Few genotype means for palmitic acid content differed significantly between the two planting dates (Table 3.8). The inconsistent responses to planting dates and slight differences among locations suggest that temperature has little effect on palmitate content (Wolf et al., 1982; Dornbos and Mullen, 1992; Primomo et al., 2002).

Stearic acid content

Irrigation significantly reduced mean stearic acid content of the genotypes studied at both planting dates (Figure 3.11). Similar results were found by Dornbos and Mullen (1992) and Flagella et al. (2002). There was a significant

irrigation x location x planting date interaction as well as a significant irrigation x genotype x location interaction (Table 3.7). Table 3.9 shows the response of the different genotypes to the irrigation treatment at each location. All genotypes at each location had a consistent decrease in stearate with the irrigated treatment, but the magnitude of the response differed between genotypes and between locations. For example, MD 99-5458, a line with low palmitic and linolenic acid content, showed only a slight effect from irrigation at each location. Therefore, it was more stable for stearic acid content than other genotypes which decreased significantly more with irrigation.

Planting date significantly affected stearic acid content and showed significant interactions with genotypes and locations (Table 3.7). Most genotypes showed a decrease in stearic at the late planting date at Columbia while they showed an increase in stearate at both Portageville locations at the late planting date (Table 3.10). At Sandhills only two genotypes had a significant response to planting date, MD 99-5458 showed a significant decrease in stearic acid content in the late planting date, while Manokin showed a significant increase. From all of these complex interactions it can be concluded that temperature does not have a consistent effect on stearic acid content, or, water availability, maturity and other factors interact with temperature to affect stearic acid content. Previous studies also showed an inconsistent effect of temperature on stearic acid content (Dornbos and Mullen, 1992; Rennie and Tanner, 1989; Primomo et al., 2002).

Unsaturated fatty acids, oleate, linoleate, and linolenate

Even though oleic acid content showed no overall effect from irrigation, it showed significant genotype x irrigation and genotype x irrigation x planting date interactions (Table 3.7). The analysis of the response of each genotype to irrigation at each location and planting date combination showed that the four normal oleic genotypes (S01-9267, MD 99-5458, AG4902 and Manokin) exhibited a common response to irrigation with a significant location x planting date x irrigation interaction while the three mid-oleic genotypes (M23, Holl and CR 03-529) showed inconsistent responses to the irrigation treatment in these eight environments.

The response to irrigation of the four normal oleic acid genotypes at each location and planting date is shown in Figure 3.12. Oleate content decreased in the irrigated treatment at each location at both planting dates except at the early planting date at Portageville Loam where oleate content increased. Even though there was a trend for lower oleate content under irrigation, oleate content was significantly lower only at the Sandhills location at the early planting date (Figure 3.12). Boydak et al. (2002) and Flagella et al. (2002) previously reported decreases in oleate content under irrigation. Different weather patterns related to temperature and rainfall distribution at each of the environments may be the cause of the interactions observed.

There were significant planting date effects as well as genotype x location x planting date interactions for oleic acid content. Means at each location and planting date for each group V genotype are shown in Table 3.11. At Columbia oleic acid content was lower at the late planting for all genotypes. Oleate content

responded differently for different genotypes at the other locations (Table 3.11), but results across locations suggest high temperatures during seed filling period are associated with an increase in the oleic acid content which is consistent with other studies (Howell and Collins, 1957; Cherry et al., 1985; Wilcox and Cavins, 1992; Primomo et al., 2002).

A strong negative correlation between linoleic acid content and oleic acid content was evident. Linoleic acid content showed significant interactions similar to oleic acid content with regard to irrigation effect (Table 3.7). Therefore, the four normal oleic acid genotypes (S01-9267, MD 99-5458, AG4902 and Manokin) and the three mid-oleic acid genotypes were analyzed separately. Linoleic acid content of the normal oleic acid genotypes responded to irrigation opposite to the effect on oleic acid content (Figure 3.13). In seven of eight environments linoleate content was higher in the irrigated treatment compared to the non-irrigated treatment. At the early planting date at Portageville Loam linoleate was lower in the irrigated treatment. The three mid-oleic acid genotypes (M23, Holl and CR 03-529) showed inconsistent responses to irrigation for linoleate content. This is similar to the inconsistent response of oleate content to irrigation.

Like oleic acid, the effect of planting date and genotype x location x planting date interaction were also significant for linoleic acid content. Response of each genotype at two planting dates at each of four locations, which showed strong negative correlation with responses on oleic acid content, are shown in Table 3.12.

Irrigation produced a significant increase in the linolenic acid content of the different genotypes, which is in agreement with results reported by Boydak et al. (2002). This effect was consistent across locations and planting dates as shown by their respective non-significant interactions (Table 3.7). Table 3.13 shows the overall effect of the irrigation treatment across locations, planting dates and genotypes while Table 3.14 shows the effect of irrigation for each genotype across locations and planting dates. Linolenic acid content was higher in the irrigated treatment for all genotypes, but the magnitude of the effect varied among genotypes. Linolenic acid for the low linolenic genotypes (Holl, CR 03-529, and MD 99-5458) was less affected by irrigation. Mean linolenate content for low linolenic acid lines appeared slightly higher in irrigated treatments, but the difference was not significant (Table 3.14).

Linolenic acid content showed a significant planting date effect and a significant genotype x location x planting date interaction (Table 3.7). Means and LSDs for each genotype at each location and planting date are shown in Table 3.15. Most genotypes showed a significant increase in linolenic acid content at the late planting dates at Columbia and Sandhills whereas they showed no significant difference at both Portageville locations, except for M23 which was significantly increased at Portageville Loam. Temperature during seed filling at Portageville varied only slightly among the early and late planting dates. This is probably why mean linolenic acid content was similar for genotypes at both planting dates at Portageville. Overall, the effect of locations and planting dates showed that higher temperatures were associated with a decrease in linolenic

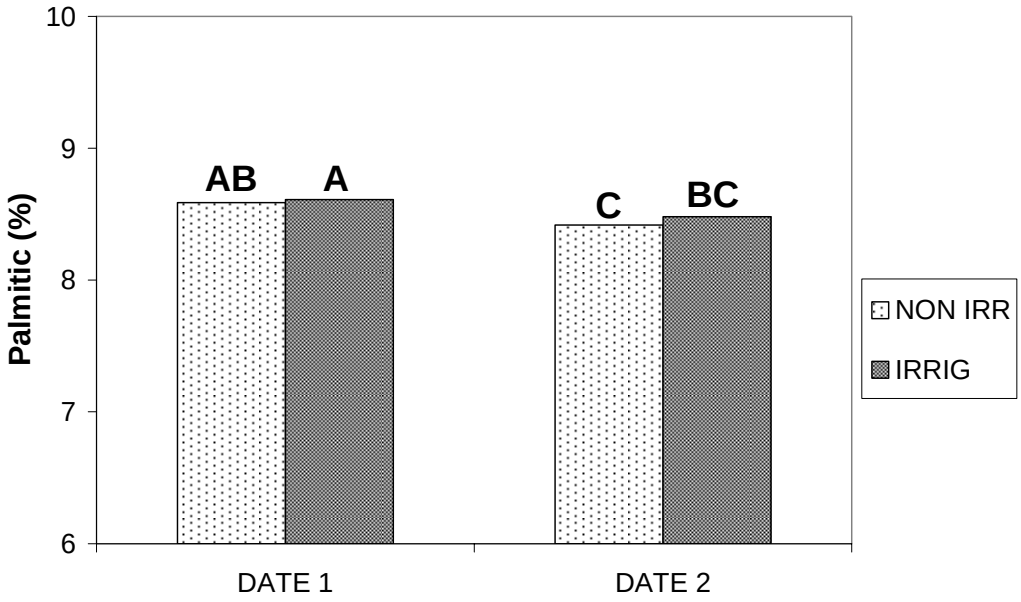
acid content, which is consistent with previous reports (Howell and Collins, 1957; Cherry et al., 1985; Wilcox and Cavins, 1992; Primomo et al., 2002).

Table 3.7. Pooled analysis of variance across four locations, two irrigation treatments, two planting dates, and seven maturity group V genotypes for palmitic, stearic, oleic, linoleic, and linolenic acids, 2004.

		Palmitic		Stearic		Oleic		Linoleic		Linolenic	
Source	**df**	**F value**		**F value**		**F value**		**F value**		**F value**	
Location (Loc)	3	9.79	**	49.81	**	87.22	**	30.2	**	190.71	**
Irrigation (Irr)	1	0.24	NS	44.13	**	0.92	NS	0.05	NS	8.9	*
Loc x Irr	3	2.23	NS	0.99	NS	1.61	NS	2.17	NS	1.41	NS
Planting Date (Date)	1	11.93	**	36.7	**	20.76	**	4.63	*	195.33	**
Loc x Date	3	1.6	NS	103.11	**	63.09	**	52.04	**	108.95	**
Irr x Date	1	0.21	NS	0.46	NS	0.02	NS	0.58	NS	1.6	NS
Loc x Irr x Date	3	1.04	NS	3.47	*	1.12	NS	0.83	NS	2.43	NS
Genotype (G)	6	2788.17	**	220.29	**	973.64	**	1303.94	**	1740.95	**
G x Loc	18	5.84	**	26.62	**	19.71	**	18.6	**	16.11	**
G x Irr	6	1.24	NS	1.58	NS	2.59	*	3.54	**	1.47	NS
G x Loc x Irr	18	2.61	**	2.37	**	1.42	NS	2.23	**	1.46	NS
G x Date	6	5.36	**	3.41	**	21.28	**	27.42	**	6.69	**
G x Loc x Date	18	2.11	**	7.62	**	5.35	**	6.2	**	5.94	**
G x Irr x Date	6	2.66	*	1.76	NS	2.49	*	2.6	*	2.15	NS
G x Loc x Irr x Date	18	1.32	NS	1.82	*	1.88	*	2.24	**	1.85	*
Pooled Error	170										

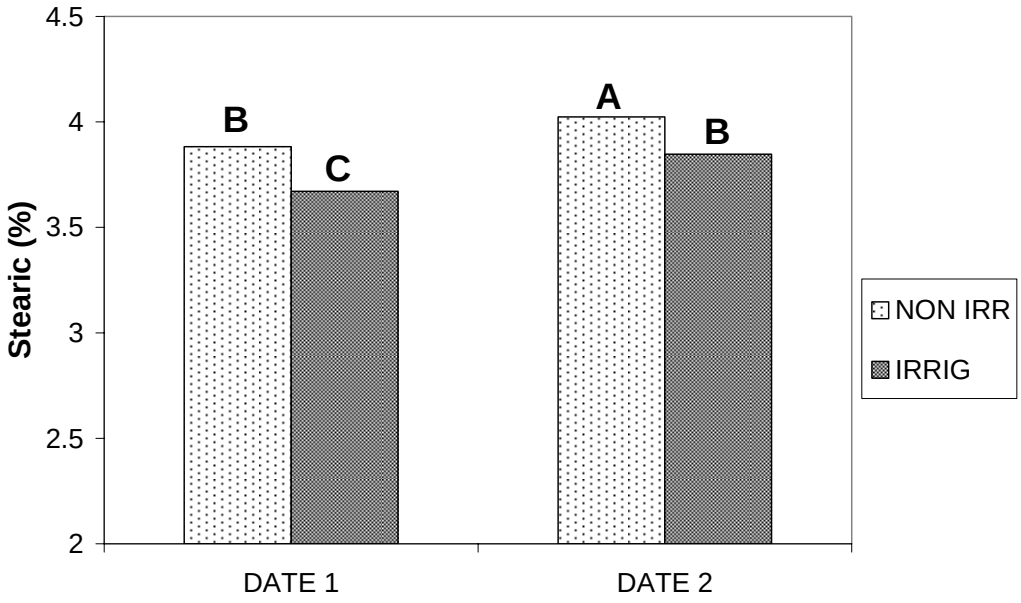
*****, ****** Significant at the 0.05 and 0.01 probability levels, respectively. NS = not significant.

Figure 3.10. Effect of irrigation on mean palmitic acid content (%) averaged across seven maturity group V genotypes and four locations at each of two planting dates, 2004.



* Means with the same letter do not differ at the 0.05 probability level.

Figure 3.11. Effect of irrigation on mean stearic acid content (%) averaged across seven maturity group V genotypes and four locations at each of two planting dates, 2004.



* Means with the same letter do not differ at the 0.05 probability level.

Table 3.8. Mean palmitic acid content (%) for seven maturity group V genotypes at two planting dates at each of four locations, 2004.

	Columbia		Port. Loam		Port. Clay		Sandhills		
Genotypes	Date 1	Date 2	Date 1	Date 2	Date 1	Date 2	Date 1	Date 2	LSD
S01-9267	4.4	4.3	4.1	4.1	5.0	4.5	4.4	4.4	0.8
M 23	8.7	8.8	9.7	9.0	10.2	9.5	9.8	9.7	0.5
Holl	8.6	8.5	9.5	8.7	9.9	9.2	10.1	9.6	0.6
CR 03-529	10.9	10.5	10.6	10.6	10.8	11.3	11.2	11.3	0.5
MD 99-5458	4.0	4.3	3.8	3.7	4.0	4.1	3.9	4.0	0.4
AG 4902	10.3	10.1	10.4	10.6	9.9	10.6	10.9	10.9	0.6
Manokin	11.2	10.4	11.3	10.9	11.7	11.2	11.8	11.9	0.7

Table 3.9. Mean stearic acid content (%) for seven maturity group V genotypes with and without irrigation at each of four locations, 2004.

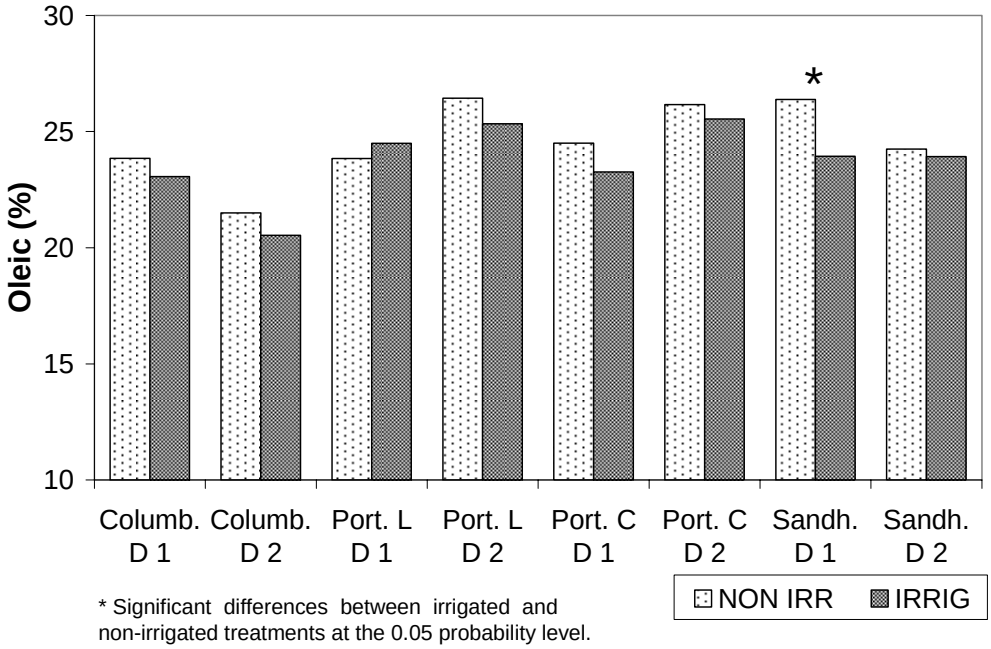
	Columbia			Port. Loam			Port. Clay			Sandhills		
Genotypes	Non Irrig.	Irrig.	Diff.	Non Irrig.	Irrig.	Diff.	Non Irrig.	Irrig.	Diff.	Non Irrig.	Irrig.	Diff.
S01-9267	3.5	3.3	-0.2	3.0	3.0	0.0	3.5	2.9	-0.6 **	2.5	2.3	-0.2
M 23	4.1	4.0	-0.1	3.9	3.6	-0.3 **	3.8	3.6	-0.2	2.9	2.7	-0.3 *
Holl	4.1	4.1	-0.1	3.8	3.6	-0.2	3.8	3.5	-0.4 *	2.9	2.8	-0.1
CR 03-529	6.0	5.7	-0.3 *	4.6	4.5	-0.1	4.2	4.2	0.0	3.5	3.0	-0.5 **
MD 99-5458	3.9	3.9	-0.1	4.1	4.1	0.0	4.1	4.0	-0.1	3.6	3.6	0.0
AG 4902	5.0	4.8	-0.2	4.0	4.0	0.0	4.0	3.9	-0.1	3.6	3.5	-0.1
Manokin	4.9	4.0	-0.9 **	4.5	4.3	-0.2	4.6	4.4	-0.1	4.1	4.1	0.0

*, ** Significant at the 0.05 and 0.01 probability levels, respectively.

Table 3.10. Mean stearic acid content (%) for seven maturity group V genotypes at two planting dates at each of four locations, 2004.

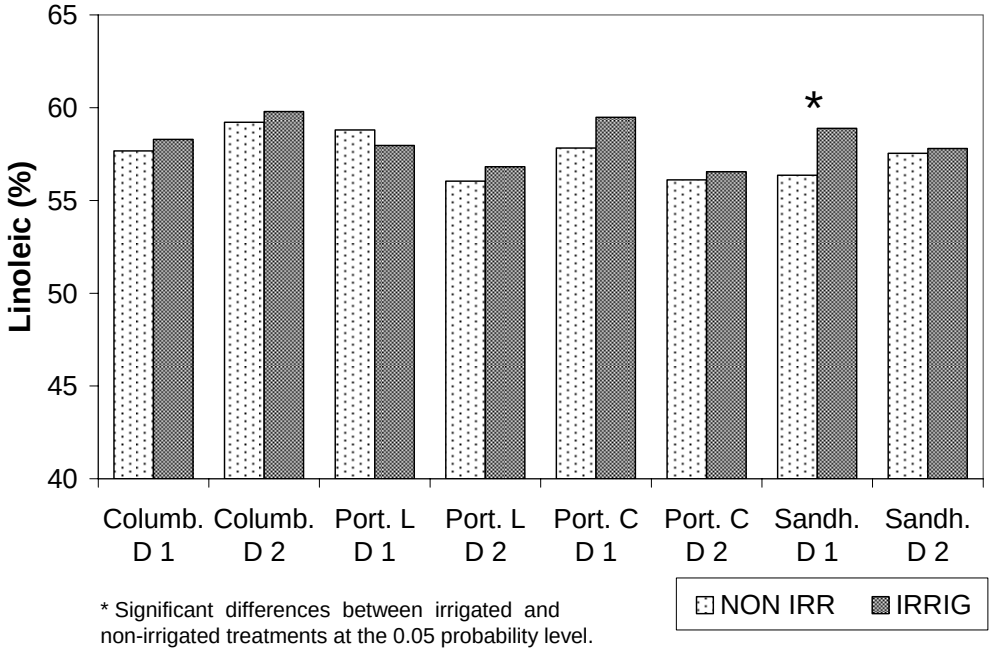
	Columbia		Port. Loam		Port. Clay		Sandhills		
Genotypes	Date 1	Date 2	Date 1	Date 2	Date 1	Date 2	Date 1	Date 2	LSD
S01-9267	3.7	3.0	2.6	3.4	3.1	3.3	2.3	2.5	0.4
M 23	4.3	3.8	3.2	4.3	3.5	4.0	2.7	2.9	0.3
Holl	4.6	3.5	3.3	4.1	3.4	3.8	2.7	3.1	0.5
CR 03-529	6.2	5.4	4.3	4.8	3.9	4.5	3.3	3.2	0.4
MD 99-5458	4.3	3.5	3.7	4.5	3.8	4.3	3.9	3.3	0.3
AG 4902	4.8	5.0	3.9	4.1	3.9	4.1	3.5	3.6	0.4
Manokin	4.4	4.4	4.2	4.6	4.3	4.6	3.8	4.3	0.3

Figure 3.12. Effect of irrigation on oleate content averaged over four normal oleic genotypes in eight environments (two planting dates, D, at each of four locations), 2004.



* Significant differences between irrigated and non-irrigated treatments at the 0.05 probability level.

Figure 3.13. Effect of irrigation on linoleate content averaged over four normal oleic genotypes in eight environments (two planting dates, D, at each of four locations), 2004.



* Significant differences between irrigated and non-irrigated treatments at the 0.05 probability level.

Table 3.11. Mean oleic acid content (%) for seven maturity group V genotypes at two planting dates at each of four locations, 2004.

	Columbia		Port. Loam		Port. Clay		Sandhills		
Genotypes	Date 1	Date 2	Date 1	Date 2	Date 1	Date 2	Date 1	Date 2	LSD
S01-9267	23.8	20.4	29.0	27.9	27.7	28.2	28.1	26.2	2.5
M 23	48.9	39.4	41.5	48.4	41.9	48.0	46.6	44.8	3.9
Holl	47.6	32.8	42.7	46.4	44.2	45.7	39.7	43.1	4.8
CR 03-529	31.9	23.3	42.8	39.9	43.3	38.1	49.8	37.4	2.5
MD 99-5458	23.1	20.2	23.5	27.9	24.1	28.9	26.7	28.6	2.6
AG 4902	26.2	23.2	23.1	25.0	21.9	24.0	25.2	21.3	2.8
Manokin	20.7	20.2	21.0	22.7	21.8	22.4	19.8	22.1	1.4

Table 3.12. Mean linoleic acid content (%) for seven maturity group V genotypes at two planting dates at each of four locations, 2004.

	Columbia		Port. Loam		Port. Clay		Sandhills		
Genotypes	Date 1	Date 2	Date 1	Date 2	Date 1	Date 2	Date 1	Date 2	LSD
S01-9267	60.6	62.0	57.3	57.6	58.0	57.5	58.1	59.0	2.1
M 23	31.6	38.4	38.8	32.5	38.7	32.5	35.2	35.9	3.6
Holl	35.0	44.4	40.9	37.0	38.9	37.4	41.7	39.4	4.2
CR 03-529	47.2	55.8	38.8	41.5	38.7	42.9	32.7	44.7	2.3
MD 99-5458	64.1	66.3	65.0	60.0	64.4	58.9	61.9	61.7	2.5
AG 4902	51.7	53.8	55.0	53.2	56.7	54.2	52.9	55.3	2.5
Manokin	55.6	55.9	56.2	54.9	55.6	54.7	57.5	54.8	1.2

Table 3.13. Effect of irrigation on linolenic acid content (%) averaged across two planting dates at each of four locations and seven maturity group V genotypes, 2004.

Treatments	Linolenic Acid Content (%)
Non Irrigated	5.7
Irrigated	5.9
LSD (0.05)	0.12

Table 3.14. Effect of irrigation on linolenic acid content (%) averaged across two planting dates at each of four locations for each of seven maturity group V genotypes, 2004.

	Treatments		
Genotypes	Non Irrigated	Irrigated	Difference
S01-9267	7.1	7.6	0.5 *
M 23	6.5	6.7	0.2
Holl	3.9	4.0	0.1
CR 03-529	3.5	3.5	0.0
MD 99-5458	4.1	4.1	0.0
AG 4902	7.6	7.7	0.1
Manokin	7.2	7.5	0.3 *

* Significant at the 0.05 probability level.

Table 3.15. Mean linolenic acid content (%) for seven maturity group V genotypes at two planting dates at each of four locations, 2004.

	Columbia		Port. Loam		Port. Clay		Sandhills		
Genotypes	Date 1	Date 2	Date 1	Date 2	Date 1	Date 2	Date 1	Date 2	LSD
S01-9267	7.4	10.2	7.2	7.0	6.2	6.5	6.5	7.9	0.7
M 23	6.5	9.3	6.8	5.8	5.8	6.0	5.7	6.7	0.5
Holl	4.1	5.8	3.5	3.8	3.6	3.8	3.3	3.7	0.4
CR 03-529	3.8	4.8	3.4	3.3	3.3	3.2	3.0	3.5	0.3
MD 99-5458	4.5	5.7	4.0	3.9	3.8	3.8	3.6	3.9	0.3
AG 4902	7.1	7.9	7.7	7.1	7.7	7.2	7.4	9.2	0.7
Manokin	8.0	9.0	7.3	6.9	6.5	7.0	7.0	7.0	0.6

Chapter IV

Phenotypic Stability Analysis

Temperature during seed filling affects fatty acid composition of soybean seed oil (Howell and Collins, 1957; Wolf et al., 1982; Rennie and Tanner, 1989; Dornbos and Mullen, 1992). Therefore, it is important to determine stability of fatty acids across environments among genotypes differing in fatty acid profile. The stability of fatty acid composition of 17 genotypes was assessed across environments using the slope of the linear regression of fatty acid values on average temperature during the final 30 days until maturity (seed filling period of maximal lipid deposition). Higher slopes for each genotype indicate low stability and lower slopes indicate high stability across environments for a particular fatty acid.

Other studies addressing phenotypic stability have used the environmental index as a quantitative measure of the environment (Finlay and Wilkinson, 1963; Eberhart and Russell, 1966; Primomo et al., 2002). The environmental index is obtained from averaging the values of all genotypes in that particular environment. When the response of all genotypes to environments is not highly correlated, however, the use of such index could lead to misinterpretation of the regression coefficients (Carver et al., 1986). In our study, responses to environments differed substantially among genotypes. Thus, the use of average temperature provides an environmental measure that is independent of the genotypes used in the study.

In this study, 10 growing environments from the combination of five locations each with two planting dates were used to obtain stability parameters by regression analysis. Since genotypes varied widely for maturity, the average temperature prior to maturity (R8) was calculated separately for each genotype by averaging the mean daily temperature for the final 30 days to its maturity date. Thus, a comparison among genotypes of different maturity may be performed within the range of average temperatures during the final 30 days to maturity.

A stability analysis was performed for the unsaturated fatty acids oleic, linoleic and linolenic acid, which were consistently influenced by temperature. The fatty acid content for stearic and palmitic acids was inconsistent under different temperature regimes and not analyzed for stability (Wolf et al., 1982; Primomo et al., 2002).

Oleic, linoleic and linolenic stability coefficients and corresponding coefficients of determination (r^2) for each genotype are shown in Table 4.1. Differences in stability coefficients show that genotypes differing in fatty acid profile differ in their response to temperature changes. Since oleic and linoleic acids show a strong negative correlation (Table 4.2), results for oleic acid content were opposite of linoleic acid content. Oleic and linolenic acid levels are important in improving functionality of the soybean oil (Wilson, 2004). Therefore, the discussion of the stability analysis will focus primarily on stability of these two unsaturated fatty acids.

In general, oleic acid stability values were positive which shows that oleic acid content increased with an increase in temperature. Oleic acid content for 16

of the 17 genotypes evaluated increased from 0.05% to 3.35% per ºC increase in average temperature during the final 30 days of seed filling. The coefficient of determination provides a measure of the proportion of variability in fatty acid content accounted for by temperature changes. The most unstable genotypes such as mid-oleic lines N97-3363-4 or N98-4445A had higher b values (2.27 and 3.35) and higher r^2 indicating a high proportion of the variability for oleic acid content can be attributed to temperature changes. On the other hand, in the most stable genotypes, such as AG 4902, temperature accounted only for a small proportion of the variability in oleic acid content.

Genotypes differed not only in their mean oleic acid content, but they also differed in the level of expression of this trait across environments, resulting in differences in stability. The genotypes with higher mean oleic acid content, especially N98-4445A and N97-3363-4, (Table 2.1) generally had higher stability coefficients than normal oleate lines (Table 4.1); thus, they were less stable across environments. Warmer environments would enhance expression of high oleic content in mid-oleic genotypes with low stability. On the other hand, the genotypes Holl and M23 with relatively high mean oleic acid content (40-50%) were more stable across environments with stability coefficients of 0.68 and 0.14, respectively (Table 4.1). These or other mid-oleic genotypes with genetic mechanisms for greater stability across environments would be favored in breeding programs to develop mid-oleic genotypes which are less influenced by temperature. Selection for stability of oleic acid content in a breeding program would be useful to develop high oleic acid lines with good adaptation to a wide

array of environments and especially to cooler growing conditions where they would still have high expression.

Figures 4.3 and 4.4 show linear regressions of oleate content on mean temperature across environments for two mid-oleic acid genotypes and a widely grown cultivar. Genotypes M23 and N97-3363-4 (Figure 4.3) exhibited a cross-over interaction in their oleate content linear response to the environment. Both M23 and AG4902 showed non-significant regressions of oleic acid content on mean temperature; therefore, temperature had little effect, and factors other than temperature were responsible for variability in oleic acid content in these genotypes across environments. Nevertheless, mean oleic acid levels of M23 and AG4902 were stable across environments. N97-3363-4 was very unstable across environments for oleic acid content as can be seen from its significant linear regression on temperature and a high regression coefficient of 3.35 (Figure 4.3). A similar cross-over interaction was found among genotypes N98-4445A and Holl (Figure 4.4). Both Holl and Manokin showed significant regressions of oleic acid content on temperature with stability coefficients of 0.68 and 0.19 respectively, as compared to the high stability coefficient of 2.59 for N98-4445A.

Holl is derived from M23 and both lines likely share the same gene for high oleic acid content (Rahman et al., 2001). N98-4445A and N97-3363-4 are also genetically related and may share one or more genes for high oleic acid. N98-4445A and N97-3363-4 have high stability coefficients, whereas the stability coefficients from M23 and Holl are low. It is not known whether these different germplasm sources of high oleic acid affect the same or different enzymes in the

fatty acid pathway. It is possible that they affect different enzymes and that these enzymes are affected differentially by temperature changes. It is also possible that mutations in these genotypes affect the same enzyme or enzymes, while they may differ at other loci that regulate the activity of these enzymes.

Linolenic acid stability coefficients are negative since linolenic acid content decreased as temperature increased (Table 4.1). The stability coefficients for the 17 genotypes ranged from 0.02 to 0.51 percent decrease in linolenate content per ºC increase in temperature. Most genotypes showed high coefficients of determination of their regressions of linolenic acid content on temperature; therefore, temperature accounted for a high proportion of the variability in linolenate content.

Genotypes with reduced linolenic acid had more stable expression than did genotypes with normal linolenic content (Figure 4.2). Linolenic acid in low linolenic lines was influenced much less by changes in temperature as compared to normal linolenic or other genotypes with altered palmitic or oleic acid content in these studies. Stability regressions for linolenic acid of cultivar DKB 38-52 and three reduced linolenic genotypes (MD 00-6605, IA 3018, and IA 3017) are compared in Figure 4.5. In general, genotypes with lower mean linolenate content were more stable than genotypes with higher linolenate content. IA 3017 with about 1% mean linolenic acid content was the most stable genotype with a stability coefficient of -0.02.

Figure 4.6 shows the increased stability of the two reduced linolenate genotypes S01-9370 and CR03-529 compared to the regular cultivar Manokin.

These results show that breeding to decrease the linolenate content would enhance stability of low linolenic acid levels over different growing conditions.

Table 4.1. Stability coefficients for oleic, linoleic and linolenic acid contents for 17 genotypes calculated over 10 seed-filling temperature environments, 2004.

		Stability coefficients (b values)					
Genotype	Phenotype	Oleic	r^2	Linoleic	r^2	Linolenic	r^2
IA 3017	Reduced Linolenic	0.86	0.20	-0.79	0.18	-0.02	0.22
IA 3018	Reduced Linolenic	0.41	0.10	-0.26	0.05	-0.07	0.45
S01-9370	Reduced Linolenic	0.13	0.07	-0.12	0.05	-0.08	0.63
C 1943	Reduced Palmitic	2.27	0.64	-1.59	0.41	-0.51	0.79
S01-9267	Reduced Palmitic	0.65	0.53	-0.35	0.36	-0.25	0.48
C 1727	Elevated Palmitic	0.05	0.00	0.30	0.16	-0.31	0.54
M 23	Mid Oleic	0.14	0.01	0.12	0.01	-0.26	0.50
N 97-3363-4	Mid Oleic & Red. Linolenic	3.35	0.74	-2.69	0.66	-0.17	0.78
N 98-4445A	Mid Oleic & Red. Linolenic	2.59	0.63	-2.16	0.56	-0.14	0.61
Holl	Mid Oleic & Red. Linolenic	0.68	0.22	-0.37	0.08	-0.18	0.64
CR03-529	Mid Oleic & Red. Linolenic	1.89	0.87	-1.63	0.64	-0.16	0.78
MD 00-6605	Red. Palm. & Red Linolenic	1.12	0.52	-0.93	0.41	-0.12	0.64
MD 99-5458	Red. Palm. & Red Linolenic	0.48	0.31	-0.28	0.14	-0.17	0.73
DKB 38-52	Regular Cultivar	0.19	0.02	0.03	0.00	-0.19	0.44
MPV 457	Regular Cultivar	-0.10	0.01	0.37	0.09	-0.17	0.34
AG 4902	Regular Cultivar	0.09	0.01	0.08	0.01	-0.10	0.19
Manokin	Regular Cultivar	0.19	0.15	-0.04	0.01	-0.22	0.68

Table 4.2. Correlation coefficients and p-values on a plot basis for five fatty acids calculated over 10 seed-filling temperature environments, 2004 (n=841).

	Stearic	Oleic	Linoleic	Linolenic
Palmitic	0.10	-0.20	-0.10	0.09
	0.005	<.0001	0.004	0.009
Stearic		-0.06	-0.02	-0.07
		0.106	0.576	0.037
Oleic			-0.93	-0.42
			<.0001	<.0001
Linoleic				0.21
				<.0001

Figure 4.1. Relationship of mean oleic acid content and oleic stability coefficient for each of 17 genotypes calculated over 10 seed-filling temperature environments, 2004.

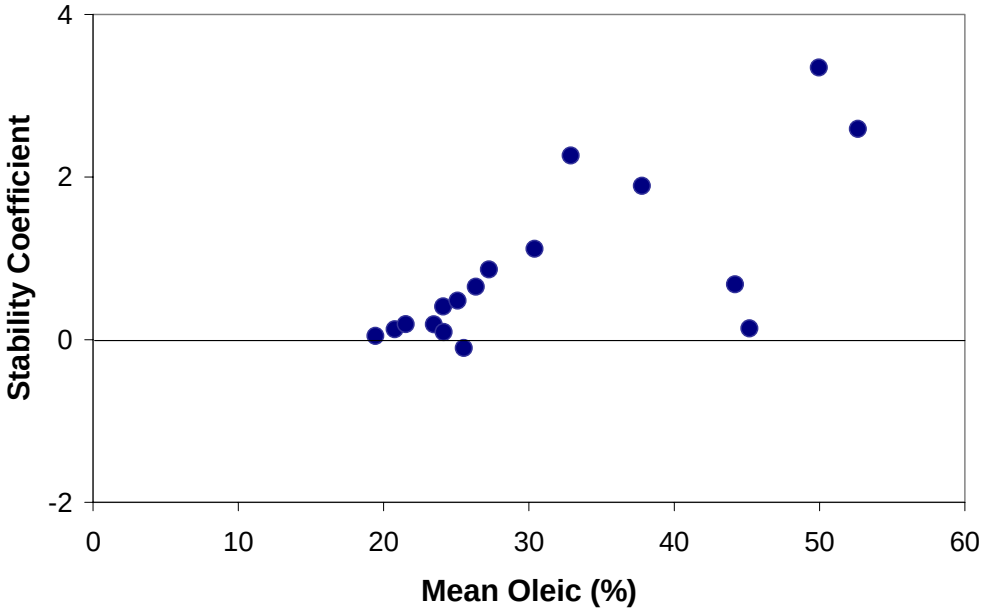


Figure 4.2. Relationship of mean linolenic acid content and linolenic stability coefficient for each of 17 genotypes calculated over 10 seed-filling temperature environments, 2004.

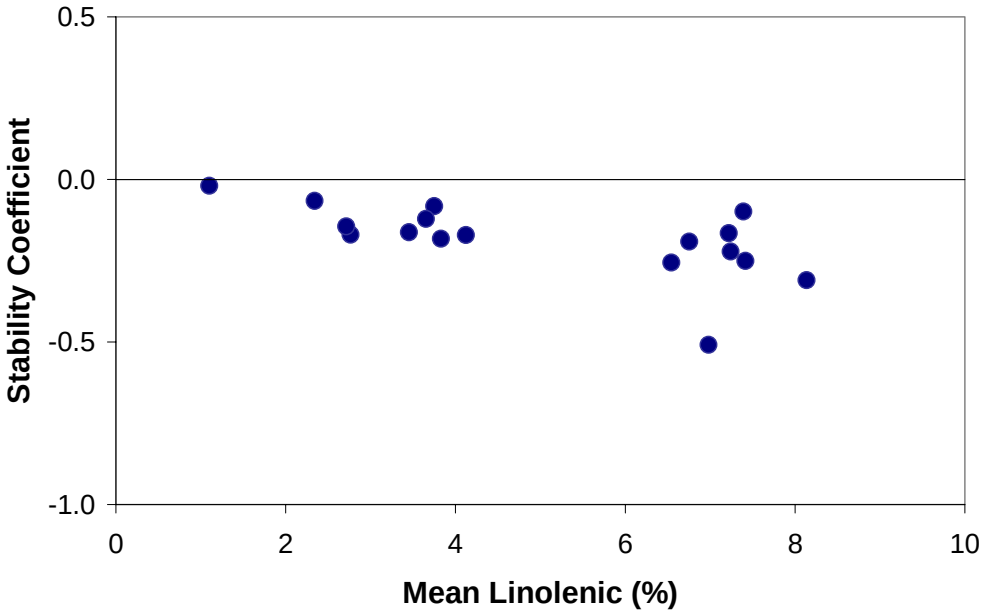


Figure 4.3. Regressions of oleate content on mean temperature for genotypes N97-3363-4, M23 and AG 4902 calculated over 10 seed-filling temperature environments, 2004.

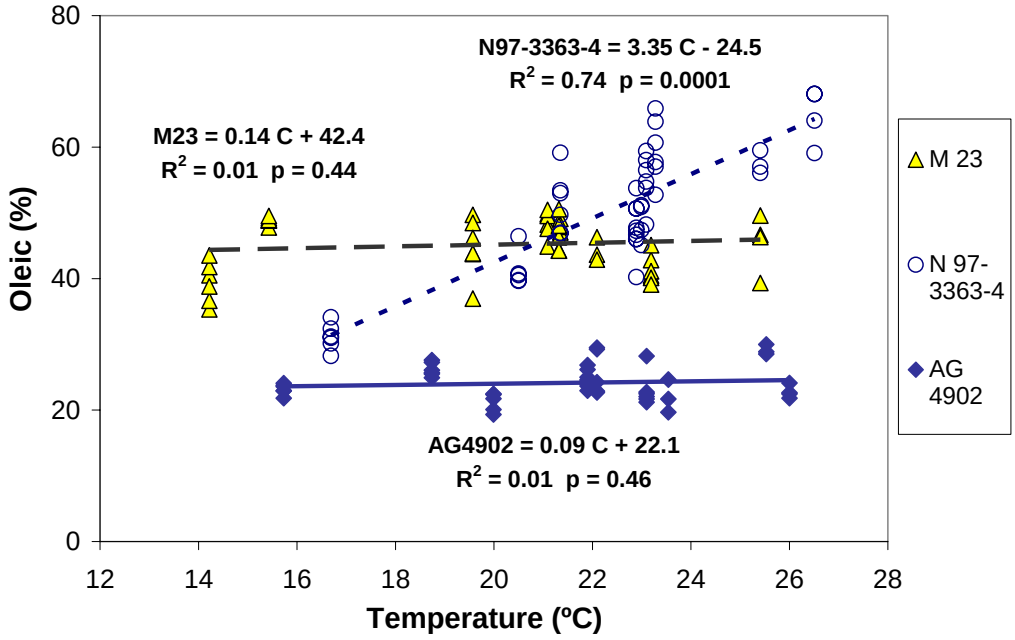


Figure 4.4. Regressions of oleate content on mean temperature for genotypes N98-4445A, Holl and Manokin calculated over 10 seed-filling temperature environments, 2004.

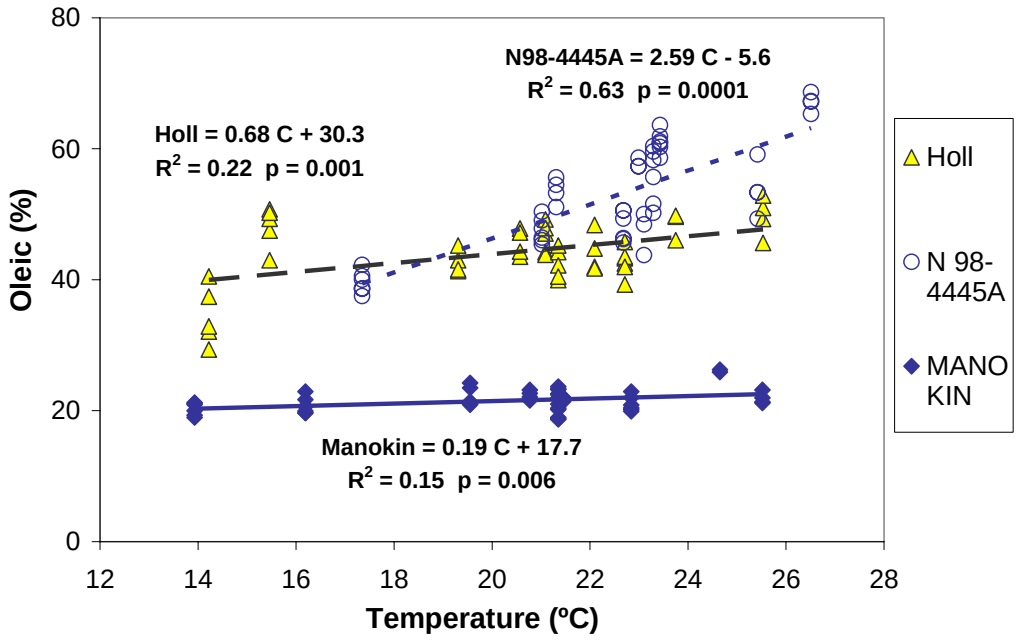


Figure 4.5. Regressions of linolenate content on mean temperature for genotypes DKB 38-52, MD 00-6605, IA 3018 and IA 3017 calculated over 10 seed-filling temperature environments, 2004.

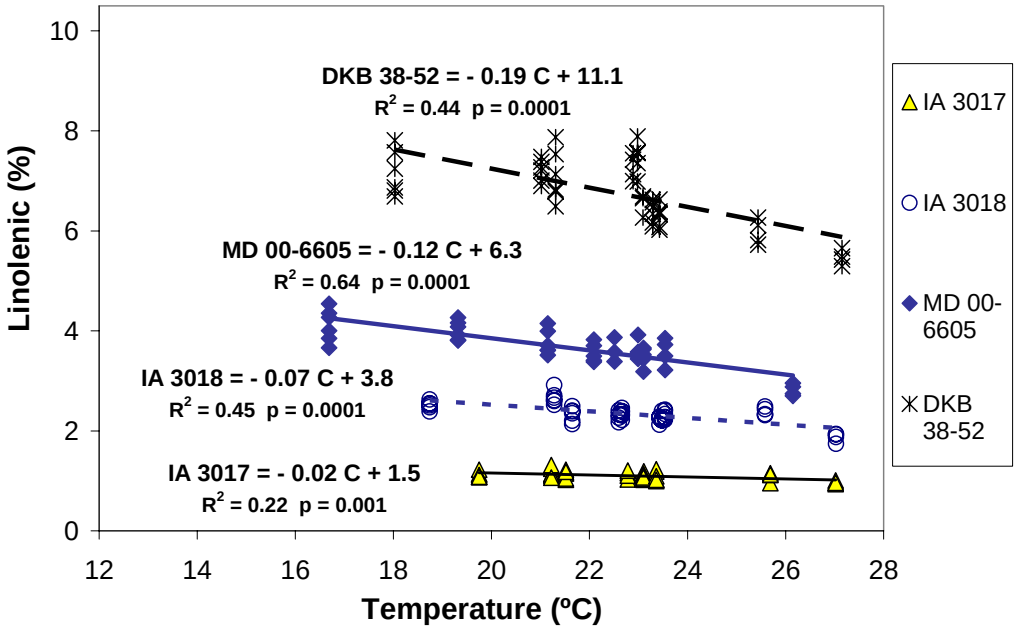
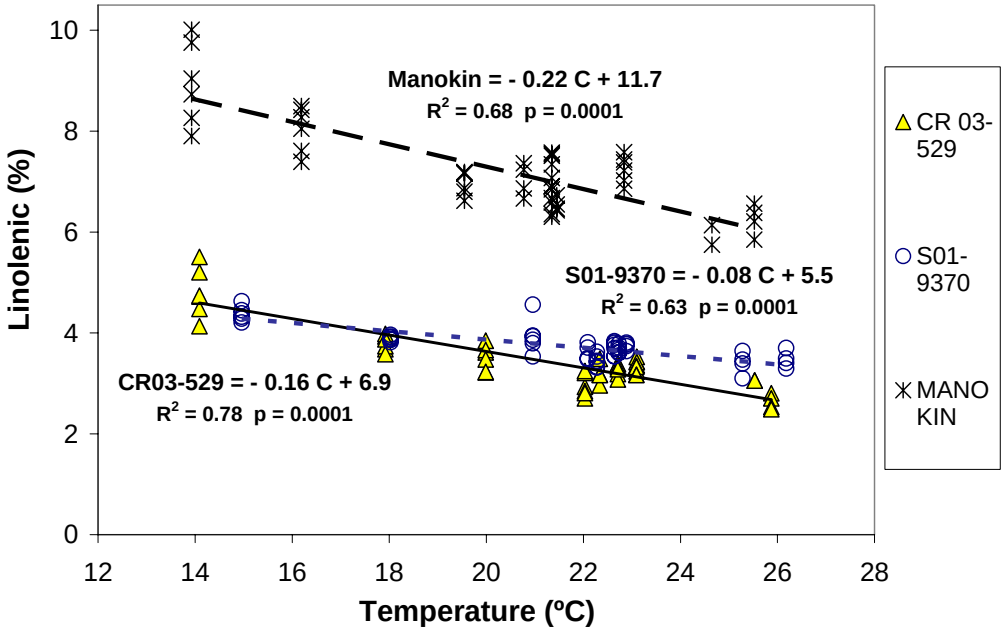


Figure 4.6. Regressions of linolenate content on mean temperature for genotypes Manokin, S01-9370 and CR03-529 calculated over 10 seed-filling temperature environments, 2004.



Chapter V

Nodal Position Effect on Fatty Acid Composition

Four genotypes were selected to study the effect of the plant nodal position on fatty acid composition. Single plants of genotypes N 97-3363-4 (mid-oleic & reduced linolenic), CR 03-529 (mid-oleic & reduced linolenic), MD 00-6605 (reduced palmitic & reduced linolenic), and AG 4902 (regular cultivar) were collected at Columbia at the early and late planting dates. Pods from each node on the main stem were collected and put in separate envelopes for fatty acid analysis. Since most of the plants had between 15 and 17 nodes on the main stem, the 15 uppermost nodes of the plant were analyzed for fatty acid composition by using two or three seeds from each node. Nodes were counted from top to bottom; therefore, node 1 was the uppermost node of the plant. Two plants from each genotype at each of the early and late planting dates were used for the analysis.

To increase the consistency of the data the nodes were grouped for statistical analysis. Results from three contiguous nodes were averaged to form a group. Node group 1 was the average of nodes 1 to 3, node group 2 was the average of node 4 to 6 and so on; thus, the plant was divided into 5 node groups from a total of 15 nodes. A regression analysis on node-group position and planting date was performed using the PROC REG procedure from SAS.

Node-group position showed significant effects for palmitic and linolenic acid contents, whereas nodal position had little effect on the content of stearic, oleic and linoleic acids.

Palmitic acid content increased significantly from the top to the bottom of the plant. Since planting date had no significant effect on palmitic acid content, planting dates were combined to calculate regressions for each genotype (Figures 5.1 to 5.4).

Linolenic acid content of seed oil at both planting dates decreased significantly from the top to the bottom of the plant for genotypes N 97-3363-4, CR 03-529, and MD 00-6605 (Figures 5.5 to 5.7). Linolenic acid content from seed harvested from various nodes showed no significant difference in AG 4902 (Figure 5.8). The effect of planting date on linolenic acid content was significant and showed no interaction with nodal position.

Temperature is an important factor that may influence the linolenic and palmitic acid content of seed harvested from different nodes on the plant. Pods lower in the plant develop earlier in the season and therefore are exposed to higher temperatures during seed filling than pods that develop later in the season. Canopy shading would have the opposite effect because of cooler temperatures down in the canopy due to shading.

Spectral quality of radiation has been shown to affect the fatty acid composition under controlled conditions (Holden et al, 1994; Britz and Cavins, 1993). Linoleic and linolenic acid contents in seed oil increased when soybean plants developed under simulated shade with the use of reduced blue or increased far red lights. Holden et al. (1994) found the activity of the endoplasmic reticulum omega-6 desaturase was regulated by spectral quality, while the chloroplast omega-6 and omega-3 desaturase activities did not appear to be

regulated by spectral quality. They suggested that phytochrome and blue light photoreceptors are responsible for regulation of the ER omega-6 desaturase. In this case, both the spectral quality and the photoperiod should affect the fatty acid composition of the seed oil.

In our study very short rows (61 cm) were used and some plots had thin stands due to emergence problems; therefore, canopy shading may have had less effect under these conditions than in normal populations. Pods lower in the canopy develop earlier in the season and are exposed to higher temperatures during seed fill. The effect of higher temperatures during seed fill seems to be the main factor responsible for the decrease in linolenic acid content on lower nodes observed in this study, while other factors like plant water status possibly interacting with temperature may have affected palmitic acid content in the seed oil from different nodes.

Figure 5.1. Regression of palmitic acid content on node group position combined across two planting dates at Columbia for genotype N 97-3363-4, 2004.

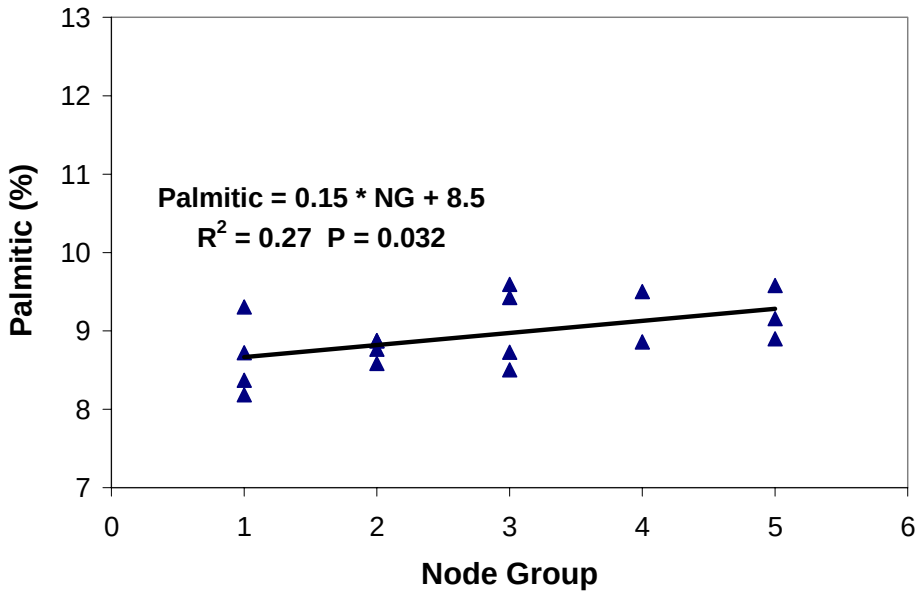


Figure 5.2. Regression of palmitic acid content on node group position combined across two planting dates at Columbia for genotype CR 03-529, 2004.

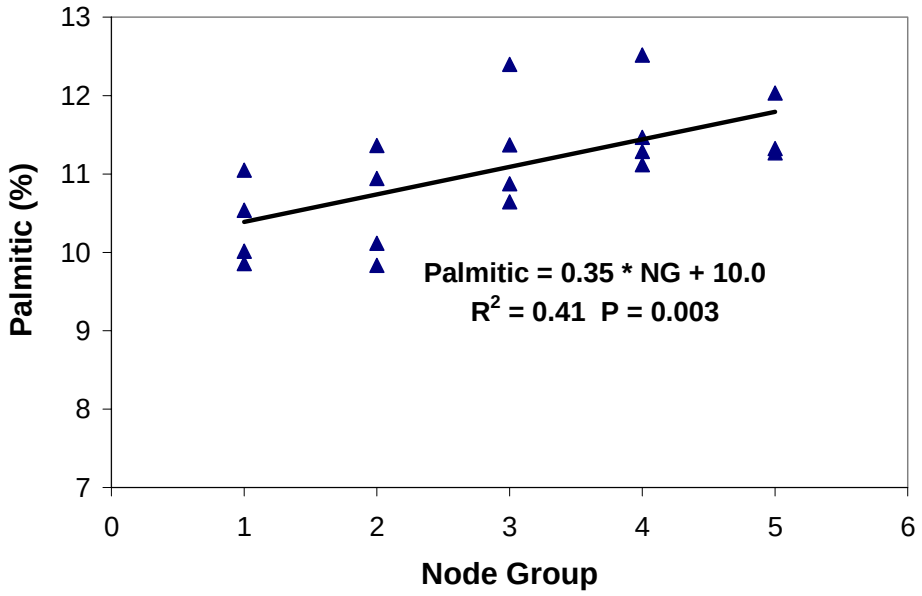


Figure 5.3. Regression of palmitic acid content on node group position combined across two planting dates at Columbia for genotype MD 00-6605, 2004.

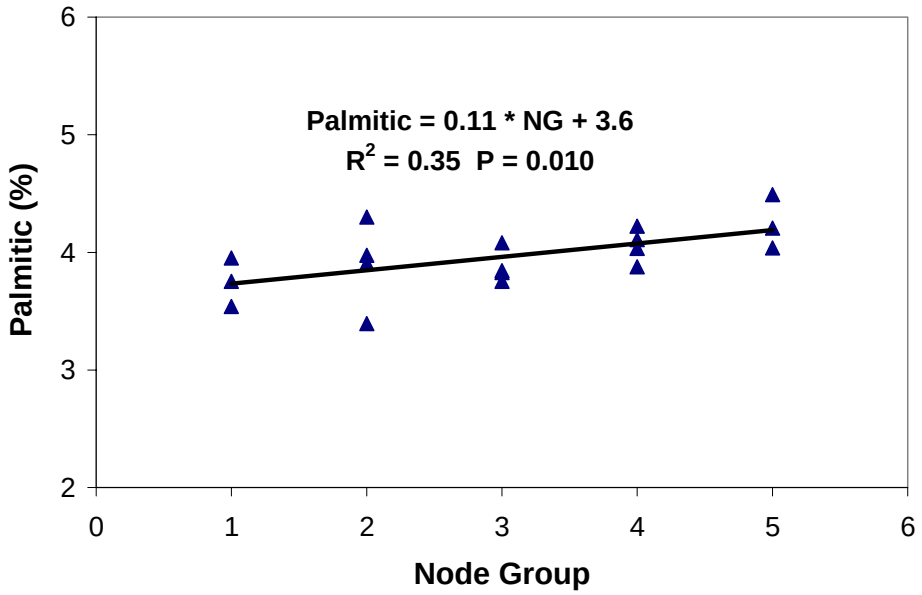


Figure 5.4. Regression of palmitic acid content on node group position combined across two planting dates at Columbia for genotype AG 4902, 2004.

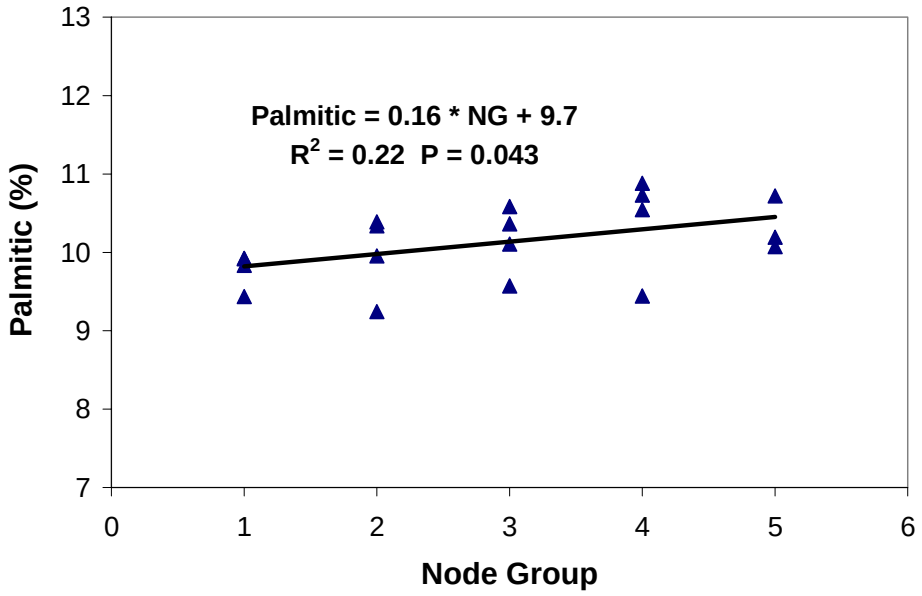


Figure 5.5. Regressions of linolenic acid content on node group position for genotype N 97-3363-4 at early and late planting dates at Columbia, 2004.

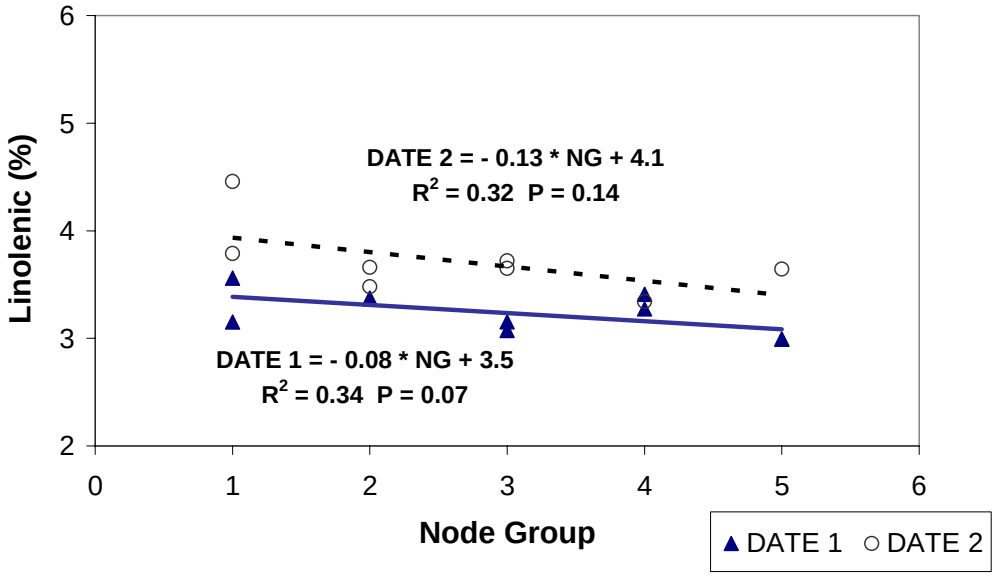


Figure 5.6. Regressions of linolenic acid content on node group position for genotype CR 03-529 at early and late planting dates at Columbia, 2004.

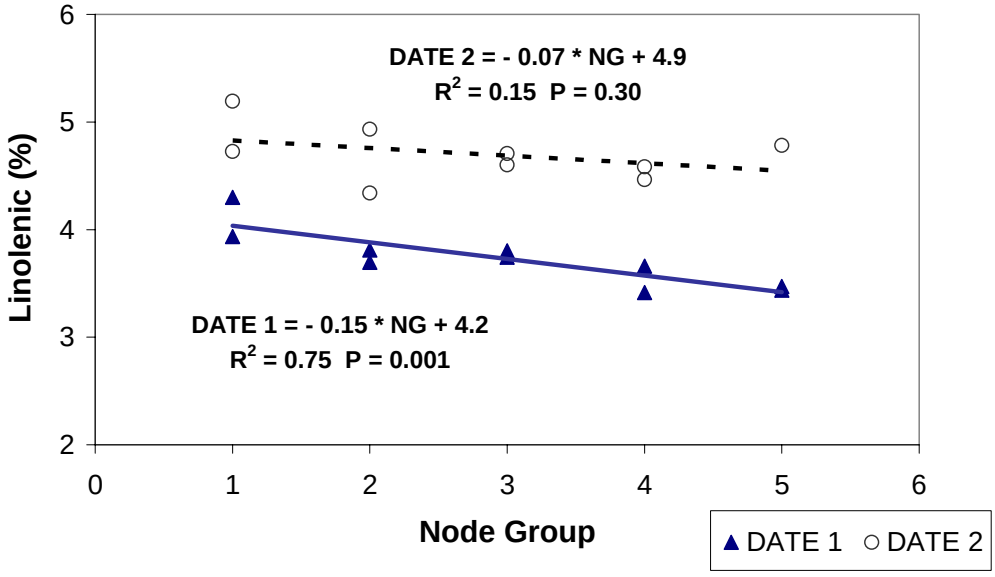


Figure 5.7. Regressions of linolenic acid content on node group position for genotype MD 00-6605 at early and late planting dates at Columbia, 2004.

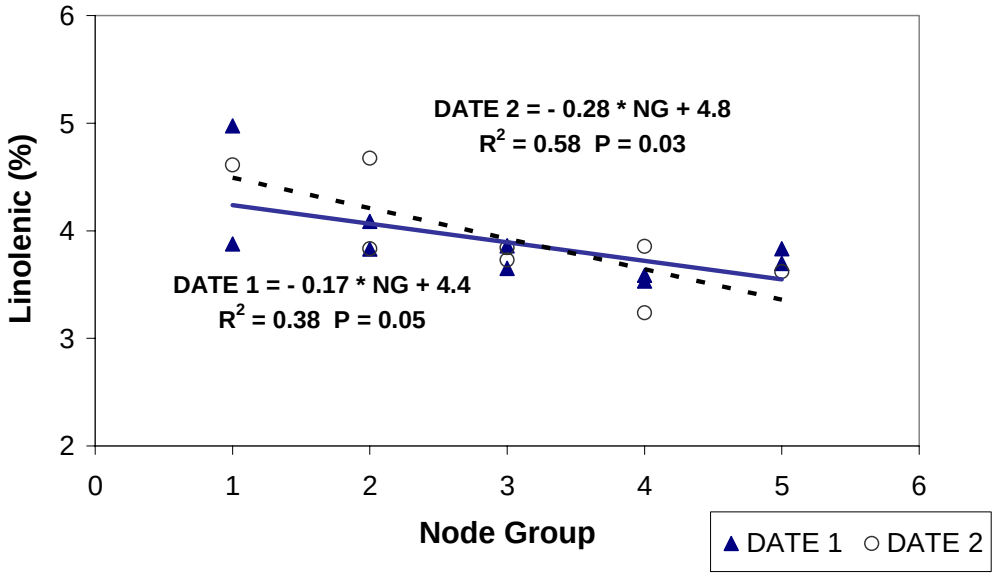
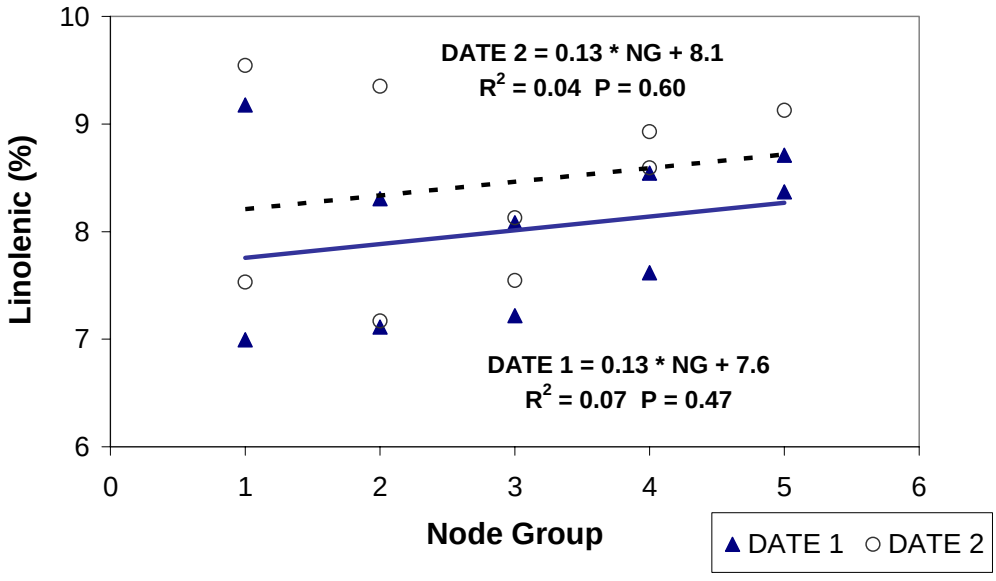


Figure 5.8. Regressions of linolenic acid content on node group position for genotype AG 4902 at early and late planting dates at Columbia, 2004.



Chapter VI

Summary and Conclusions

Irrigation affected saturated fatty acid content in soybean seed oil of 17 genotypes varying in fatty acid profile. Palmitate content was higher in irrigation treatments regardless of planting date at each location. On the other hand, stearate content in the seed oil averaged less in irrigated versus non-irrigated treatments across locations and planting dates.

There was no consistent effect of planting dates on palmitate and stearate content in our study. The response to planting dates exhibited significant interaction with locations and genotypes. Temperature and water status in the plant at the seed filling period likely interact in modifying palmitate and stearate contents in the soybean seed oil. Also, rainfall distribution during the growing season, as well as the growing stages at which the irrigation is applied may be important factors that affect the contents of both saturated fatty acids.

Unsaturated fatty acid contents were also influenced by the irrigation treatment. Oleate decreased, while linoleate and linolenate increased with irrigation. The interaction of irrigation with locations and genotypes affected both oleate and linoleate content. Differences in rainfall distribution among locations may have been an important cause of the irrigation x location interaction, whereas differences in the dates of the seed filling period among genotypes may have been partially responsible for the irrigation x genotype interaction. Linolenate content was consistently higher in irrigated versus non-irrigated treatments across locations, planting dates and genotypes. The average

increase, even though statistically significant, was only 0.07% for the maturity group III and IV genotypes and 0.17% for the maturity group V genotypes evaluated. The 2004 growing season had above average rainfall at most locations in the study; therefore, the environments in this study did not experience prolonged water deficit. Environments where genotypes are exposed to longer water deficits during certain periods of the growing season and especially during seed fill will be necessary to compare the irrigation response on those situations to the results reported in this study. The results of this study are in agreement with previous reports in which linoleic acid increases and oleic acid decreases with higher water availability in soybean (Boydak et al., 2002) and the oleic and stearic acid contents decrease and linoleic and palmitic acid contents increase under irrigation in sunflower (Flagella et al., 2002). Further studies are needed comparing how irrigation timing at different soybean growth stages affects fatty acid composition of the seed oil.

There was a significant planting date effect on the unsaturated fatty acid content, and a significant planting date x location interaction. Early planting resulted in an increase in oleic acid content and a decrease in linoleic and linolenic acid contents at Columbia, Sandhills and Stoneville. The opposite response to planting dates was found at Portageville, with a decrease in oleic acid content, and an increase in linoleic and linolenic acid content at the early planting dates. Analysis of average daily temperatures at each location and planting date showed that there was only a slight difference in temperature during seed fill between early and late planting dates at Portageville. At the other

locations the average temperatures during seed fill were 0.7 to 2.5 ºC higher at early planting dates than at late planting dates. Therefore, temperature was likely to be the main factor underlying the response of the unsaturated fatty acids to planting dates described above.

Most years, early planting dates result in higher temperatures during the seed filling period than normal or late planting dates. Therefore, early planting dates should result in seed oil with higher oleic acid and lower linolenic acid which is desirable for producing more functional oil.

A stability analysis over locations showed the contents of oleic, linoleic and linolenic were strongly correlated with average temperature during the final 30 days of seed fill. The Stoneville, MS location had the highest average temperature during seed fill and produced seed with the highest oleate and lowest linolenate contents. Conversely, Columbia had the lowest average temperatures during seed fill and produced seed with lowest oleate and highest linolenate contents in this study.

Even though temperature affected unsaturated fatty acid content on all genotypes, the magnitude of the temperature effect varied widely among genotypes. Mid-oleic acid genotypes N97-3363 and N98-4445 were less stable for oleate content across environments than genotypes with normal oleate content. Mid-oleic genotypes M23 and Holl were relatively stable across environments and therefore represent good germplasm lines to breed mid-oleic lines adapted to cooler environments or for adaptability to a wider range of growing conditions. In contrast, N98-4445A and N97-3363-4 were very

responsive to temperature changes and represent germplasm which would achieve desired high oleic acid levels when grown in warmer environments.

Breeding programs for increased oleate content in soybean should evaluate oleate content at several environments to characterize mean oleate content and stability of the oleate content across environments. In this study there was large variability in temperature during late seed filling across the ten environments. Nevertheless, the use of three environments like Portageville early planting and Columbia early and late planting provides important variation in temperature at seed filling which could be used to characterize oleate content stability of breeding lines.

Reduced linolenic acid genotypes were more stable across environments than normal genotypes. The results of this study suggest lower linolenate content is associated with higher stability across environments (Figure 4.2). Therefore, one or a few environments may be adequate to obtain a good estimate of the mean linolenic acid content and its stability across locations for a genotype.

Plant nodal position significantly affected palmitic and linolenic acid content in the seed oil. Palmitic acid content significantly increased, while linolenic acid content significantly decreased from the top to the bottom of the plant. Seasonal trends in temperature and water availability seem to be partially responsible for the nodal position effect because time of pod and seed development differs according to position on the soybean plant.

Soybean cultivars with novel fatty acid profiles, like reduced linolenate soybean, are currently available for commercial production. Cultivars with other

modified fatty acid traits (ie: mid-oleic acid) will likely be introduced for production in the near future. Knowing the best growing conditions for these novel cultivars with regard to adaptation, planting dates, response to irrigation, along with other agronomic practices will insure that the specific environment for expression of a desirable fatty acid profile of the seed oil is consistently met.

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