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EVALUATION OF FATTY ACID COMPOSITION OF COTTON GERMPLASM AND ASSOCIATION WITH COLD TOLERANCE

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Abstract

A study was designed to determine if a relationship exists between cold tolerance in cotton (*Gossypium hirsutum* L.) germplasm and the distribution of the fatty acid profile. Eleven genotypes consisting of both experimental and commercial cultivars were planted in April of 2000 and 2001 at the Texas A&M Agricultural Experiment Station in Lubbock. A warm germination test was done prior to planting to determine seed viability, and field stand counts were taken to determine the emergence rate index for comparing seedling vigor among the genotypes. Metabolic and imbibitional chilling tests were also performed on the genotypes in a growth chamber. Boll samples were taken from 2 of the 4 reps to evaluate the saturated fatty acids myristic, palmitic, and stearic, and the unsaturated fatty acids oleic, linoleic, and linolenic. The percent saturated, unsaturated, unsaturated/saturated ratio, and the oleic/linoleic ratio were also determined. In both 2000 and 2001 the oleic/linoleic ratio was significantly correlated to the Lubbock emergence rate index at the alpha level 0.1 in 2000 and at the alpha level 0.05 in 2001. Specifically, the oleic/linoleic ratio, which measures a shift within the unsaturated portion of the ratio, is negatively correlated to cold tolerance. Future research is needed to determine if the oleic/linoleic ratio could be a tool for selection in a breeding program for cold tolerance.

Introduction

Cotton (*Gossypium spp*.) is a perennial plant that originated in tropical regions (Kohel and Lewis, 1984). Plant breeders, following numerous years of breeding and selection, have developed cotton cultivars that are cultivated as annuals. The majority of these adapted cultivars permit successful culture in regions where the frost period is less than 180 days. Growers in the Southern High Plains of Texas annually plant 3.5 million acres of upland cotton, *Gossypium hirsutum L.* (Gannaway, 2002). Producers in this production region typically plant in May when soil temperature conditions are at least 15.6°C at seed depth. Ideally, producers would like to be able to plant cotton in April or even March, when conditions are less favorable. By planting earlier, producers would be able to avoid late-season weather and insect problems. In order for producers to plant earlier in the spring, cotton cultivars need to be available that can withstand colder soil temperatures. Cotton becomes inactive at temperatures below 15°C (Kohel and Lewis, 1984). Marani and Dag (1962) reported differences exist in the ability of some cotton genotypes to germinate at low temperatures. Cotton breeders have traditionally selected for cold tolerance by planting experimental genotypes early in the season and applying selection pressure for early uniform emergence. While this method of selection has worked for many years, it would benefit cotton breeders to have a better method for selecting for cold tolerance. Dogras, Dilley, and Herner (1977) cited work by Lyons, Wheaton, and Pratt (1964) that the membrane lipids of chilling-sensitive plants contain less unsaturated fatty acids than those of chilling-resistant plants. They also explain that if cellular lipids are involved in the cold sensitivity and cold acclimation of plants, knowledge of the distribution of the fatty acids in phospholipids of germinating seeds of chilling-sensitive and chilling-resistant plant species may contribute to an understanding of plant susceptibility to chilling injury (Dogras, Dilley, and Herner, 1977). This study was designed to determine if a relationship exists between cold tolerance in cotton germplasm and the fatty acid profile. If cold tolerance can be related to the distribution of the fatty acids, cotton breeders would be better able to screen for cold tolerance by knowing the fatty acid profiles of their germplasm.

Materials and Methods

Eleven genotypes listed in Table 1.were used throughout the entire study. Nine of the eleven genotypes are identified as cotton accession (CA) or El Paso source material (EPSM). These were experimental lines selected for cold tolerance by Dr. John Gannaway, Professor and Cotton Breeder at the Texas A&M Research and Extension Center. These experimental lines were chosen for variability seen within their fatty acid profiles (Gannaway, 2002). Entry numbers have been placed in parentheses at the end of each cultivar for identification, because some of the genotypic designations are identical. These genotypes with identical designations are different selections from

a population. The other two cultivars, 'Fiber Max 958' and 'Paymaster 145', are commercial cultivars that were chosen because 'Fiber Max 958' has shown rapid emergence rates under optimum conditions while 'Paymaster 145' has shown excellent emergence when soil temperatures at planting are well below optimum conditions. The eleven genotypes listed in Table 1. were planted (1½ in. depth) in four replications using a randomized complete block design at Lubbock on April 15, 2000. Prior to planting, the herbicide Prowl (1qt./acre) and 80-20-0 (lbs/acre) of fertilizer was applied to the soil. The field was pre-irrigated eight days prior to planting. In 2001, these same genotypes were planted (1½ in. depth) in four replications using a similar design at New Deal on April 11th and at Lubbock on April 19th. Prior to planting, the herbicide Prowl (1qt./acre) and 100-20-0 (lbs/acre) of fertilizer was applied to the soil. The field was pre-irrigated ten days prior to planting.

Table 1. Genotypes used in this study concerning cold tolerance.

Genotypes	Genotypes
CA 1012(1)*	CA 1044(7)
CA 1012(2)	CA 1044(8)
EPSM 1667(3)	EPSM 1224(9)
EPSM 1667(4)	Paymaster 145(10)
CA 2266(5)	FiberMax 958(11)
CA 2266(6)	

* Number in parentheses is the entry number.

Warm Germination Test

A warm germination test was performed on the seed of all genotypes shown in Table 1. prior to planting in 2001. This test was based on the standard germination procedure. Three replications of 50 seeds each were placed in wet paper towels in a germinator in which the temperature cycled for 8 hours at 30°C and 16 hours at 20°C during each 24-hour period. Germinated seeds were counted at 5 and 10 days. This was used as a measure of seed viability. The seed viability was used to determine planting rates so that each plot had similar numbers of viable seed. Therefore, stand count determinations were done on the same number of potential plants per plot. Warm germination numbers were also used as a reference for the metabolic and imbibitional germination tests.

Metabolic Chilling Tolerance Test

Three replications of 50 seeds each of the genotypes listed in Table 1. were placed in plastic boxes filled with sterilized sand previously soaked with water to field capacity. A dry layer of sterilized sand 2.5 cm deep was used to cover the seeds (Duesterhaus, 2000). This material was then placed in a growth chamber. The chamber temperature remained constant at 18°C. Stand counts were taken daily for 21 days. An "emergence percent-metabolic corrected" for each entry was calculated by adjusting the emergence percent for percent viable seed that was determined in a standard germination test. The corrected emergence percent was found by dividing each cultivar's 10-day warm germination percent into its emergence percent (Duesterhaus, 2000).

Imbibitional Chilling Tolerance Test

One hundred fifty seeds of each genotype listed in Table 1. were placed onto a polyurethane foam pad measuring 34 cm x 42 cm x 1 cm. This foam pad was rolled up and placed into a plastic tube 33.5 cm long x 5 cm in diameter. This plastic tube was then placed into a tray containing 5°C water and left until saturated. It was then placed in a refrigerator at 5°C for six hours (Duesterhaus, 2000). Three replications of 50 seeds from each of the genotypes were planted in a randomized complete block design into plastic boxes filled with sterilized sand previously soaked to field capacity. The seeds were covered with 2.5 cm of dry sand and placed into a growth chamber at 30°C for 14 days. Stand counts from each rep were taken for 14 days. An "emergence percent-imbibitional corrected" for each cultivar was calculated by adjusting the emergence percent for percent viable seed that was determined in a standard germination test. The corrected emergence percent was found by dividing the percent emergence value for each cultivar by its 10-day warm germination percentage (Duesterhaus, 2000).

Field Stand Counts

Stand counts were taken daily for 28 days in 2001 from the New Deal and Lubbock locations. These counts were used to determine the Emergence Rate Index (ERI) as follows: $\in \{E_i [(y+1)-x_i]\}$ where E_i equals accumulative emergence on day i., y equals days to final count, and x assumes the value of i. This formula was used to compare the seedling vigor among the genotypes (Duesterhaus, 2000).

Materials Evaluated for Fatty Acid Profiles

A random boll sample was taken from 2 of the 4 replications in both 2000 and 2001 to evaluate fatty acid profiles of each of the 11 genotypes. Also, the first plants to germinate and emerge were marked in the field and later harvested to evaluate their fatty acid profiles. A wide range of germplasm that includes both new and old cultivars, Russian cultivars and chemically altered mutants was also evaluated for fatty acid profile variability. These plant materials were grown at Lubbock in 2000 and 2001. The new, old, and Russian germplasm was obtained from Dr. John R. Gannaway, Professor and Cotton Breeder at the Texas A&M Agricultural Experiment Station, Lubbock, TX. The chemically altered mutants were obtained from Dr. Dick Auld, Chairman of the Plant and Soil Science Department at Texas Tech University, Lubbock, TX. All samples were ginned on a 10-saw laboratory gin (Dennis Manufacturing Co. Inc. Athens, TX). Seed samples were then delinted in concentrated sulfuric acid, rinsed with water, neutralized in sodium bicarbonate, rinsed with water and air-dried. Seed samples were pressed to obtain oil using a Carver Model C © hydraulic press from Fred S. Carver, Inc., Wabash, IN. The pressed oil was stored in a refrigerator set at 0°C. This oil was used for determination of fatty acid profiles.

Methylation Procedure for Fatty Acid Determination

The initial step in determination of fatty acid profiles is methylation. The methylation process begins with the addition of one drop of cottonseed oil into a Pierce Reactivial with approximately 1.5 ml of methylation mixture. The methylation mixture consists of 29.1 ml of 14% BF_3 in MeOH (borontrifluoride in methanol)(Sigma Chemical, St. Louis, MO) 20 ml of toluene (Fisher Scientific, Fair Lawn, NJ), and 50.9 ml of MeOH (methanol)(Fisher Scientific, Fair Lawn, NJ) (Sanders, 1980). The vial containing this mixture was block heated at 100°C for 30 minutes. The vial was shaken after the first 15 minutes. The vial was then removed from the heating block and allowed to cool. Approximately 1.5 ml of distilled water was added to the vial and the contents poured into a labeled test tube. Hexane (1.5 ml) (Fisher Scientific, Fair Lawn, NJ) was then added to the mixture. The mixture was vortexed and the top layer pipetted off and transferred to a 16 x 100 mm test tube (Sanders, 1980). This test tube and its contents were then placed under nitrogen gas to evaporate the hexane. After the hexane was evaporated, 1.0 ml of chloroform (Fisher Scientific, Fair Lawn, NJ.) was added to the residue, and 1µl was injected into a Hewlett Packard 5890 Series II gas chromatograph. The initial temperature of the gas chromatograph oven was set on 210°C, the initial detector temperature was set on 250°C, and the initial injector (7673 Hewlett Packard) temperature was set on 200°C. Fatty acid profile data were obtained from the chromatograph in approximately 30 minutes (Sanders, 1980).

Statistical Analysis

All data were analyzed using the SAS statistical procedure (SAS Institute, Inc., 2001). The GLM procedure was used for analyzing all data sets. Mean separation was accomplished by Fisher's LSD at the 5% level of significance.

Results and Discussion

Metabolic and Imbibitional Chilling Tests

When the cultivar data for the metabolic and imbibitional chilling tests were separated and ranked by LSD, the results were not comparable to the field ERI results, except that CA 1012(1) and EPSM 1224(9) and EPSM 1667(3) remained consistently in the top three. The metabolic and imbibitional germination results are listed in Table 2.

Based upon the data from the metabolic and imbibitional chilling tests, all cultivars used in this study were classified as having excellent cold tolerance. According to Duesterhaus (2000), cultivars with emergence percentages from both the metabolic and imbibitional tests of 80% or above are classified as having excellent cold tolerance. Emergence percentages 65% to 80% are ranked as having good overall cold tolerance. Emergence percentages 50% to 65% are considered having a fair overall cold tolerance, and emergence percentages of 50% and below are considered as having poor cold tolerance (Duesterhaus, 2000). The percentages for each cultivar were calculated by adjusting the emergence percent for percent viable seed that was determined in a warm germination test. The corrected emergence percentage was found by dividing the percentage emergence value for each cultivar by its 10-day warm germination percentage (Duesterhaus, 2000). The following 10-day warm germination results are listed in Table 3., and the following metabolic and imbibitional "corrected emergence percentage" results are listed in Table 4.

Table 2. Uncorrected percentage emergence for the metabolic and imbibitional tests for the 11 genotypes.

	Metabolic		Imbibitional	
Genotype	**%**	**Rank**	**%**	**Rank**
EPSM 1667(3)	91.3a†	1‡	90.7a	3‡
CA 1012(2)	90.0a	2	74.0cd	9
CA 1012(1)	90.0a	2	93.3a	2
CA 2266(5)	90.0a	2	76.7bcd	8
EPSM 1224(9)	90.0a	2	94.0a	1
EPSM 1667(4)	89.3ab	3	88.7ab	4
CA 2266(6)	88.0abc	4	87.3ab	5
CA 1044(8)	80.7abc	5	70.0de	10
CA 1044(7)	78.7bc	6	86.7abc	6
Paymaster 145(10)	77.3c	7	86.0abc	7
FiberMax 958(11)	64.7d	8	60.0e	11
Mean	84.5		82.5	

† Mean values in the same column followed by the same letter are not significantly different at the 5% level of probability.
‡Ranking based on percent germination.

Table 3. Ten-day warm germination percentage results used for seeding rate determinations in 2001.

		Germination
Genotype	**Entry #**	**(%)**
CA 1012	1	85.3bcd†
CA 1012	2	84.0cd
EPSM 1667	3	98.0a
EPSM 1667	4	87.3bc
CA 2266	5	93.3ab
CA 2266	6	92.6ab
CA 1044	7	87.3bc
CA 1044	8	72.6e
EPSM 1224	9	84.0cd
Paymaster 145	10	78.6de
FiberMax 958	11	72.6e
Mean		86.0

†Mean values in the same column followed by the same letter are not significantly different at the 5% level of probability.

Table 4. The corrected metabolic and imbibitional emergence percents used to determine the level of cold tolerance within each entry.

	Metabolic	Imbibitional
Genotype	**%**	**%**
CA 1012(1)	105.7abc†	109.8ab†
CA 1012(2)	107.6ab	89.4cde

EPSM 1667(3)	93.2cd	92.6b-e
EPSM 1667(4)	102.3a-d	101.6a-d
CA 2266(5)	96.5bcd	82.5e
CA 2266(6)	95.1bcd	94.6a-e
CA 1044(7)	95.7bcd	105.6abc
CA 1044(8)	110.8a	96.1a-e
EPSM 1224(9)	107.9ab	112.4a
Paymaster 145(10)	98.4a-d	109.7ab
FiberMax 958(11)	89.5d	83.5de
Mean	100.2	98.0

†Mean values in the same column followed by the same letter are not significantly different at the 5% level of probability.

Even though the metabolic and imbibitional test results concluded that all of the entries exhibited excellent cold tolerance, significant differences among the entries were seen in the field at both the Lubbock and New Deal locations in 2001. Entry 3 (EPSM 1667) maintained the highest emergence % and entry 8 (CA 1044) maintained the lowest emergence% at both locations. These results indicate that there was enough genetic variability within this selected group of germplasm to select for genotypes that will germinate and emerge faster under cold conditions. Cotton breeders could then select those genotypes that exhibit fast emergence and use them for cold tolerance selection.

Emergence Rate Index

New Deal soil temperatures were below 15°C for two days during the emergence period, which is considered to be a developmental threshold for cotton, in 2001. Therefore early emergence in New Deal is considered to be the best estimate of cold tolerance for the cultivars used in this study. Entry numbers 1(CA 1012), 3(EPSM 1667), and 9(EPSM 1224) ranked in the top three at both locations. Entry numbers 7, 8, 10, and 6 ranked among the bottom four in both locations, and entries 5, 4, 2, and 11 fell in the middle at both locations. The Emergence Rate Index results are listed in Table 5.

Table 5. The field emergence rate indices for New Deal and Lubbock in 2001.

Genotype	New Deal	Lubbock
EPSM 1667(3)	20,986a†	26,432a†
CA 1012(1)	20,019a	21,417ab
EPSM 1224(9)	18,463ab	18,204bc
FiberMax 958(11)	16,207bc	14,528bcd
CA 2266(5)	15,216bcd	13,818cd
EPSM 1667(4)	14,773cd	14,710bcd
CA 1012(2)	14,144cd	15,257bcd
CA 2266(6)	14,049cd	13,398cd
Paymaster 145(10)	12,919cde	12,718cd
CA 1044(7)	11,882de	10,854d
CA 1044(8)	10,497e	10,687d
Mean	15,377	15,638

†Mean values in the same column followed by the same letter are not significantly different at the 5% level of probability.

Fatty Acid Profile Results

Five major fatty acids (myristic, palmitic, stearic, oleic, and linoleic) received major emphasis in this study with the percentage of each being determined and subjected to statistical analyses. In addition, the total saturated and unsaturated fatty acid content, the unsaturated to saturated fatty acid ratio, and the oleic to linoleic fatty acid ratio were analyzed. Fatty acid profile analysis was performed only on seed harvested from the Lubbock location. Seed from the New Deal location was lost to severe weather conditions. The fatty acid profile results for 2000 and 2001 are listed in the following (Tables 6,7,8,and 9.)

Table 6. The fatty acid profiles of lipids extracted from cottonseed expressed as percent of total fatty acid of the 11 genotypes grown in Lubbock in 2000.

Genotype	14:0	16:0	18:0	18:1	18:2
CA 1012(1)	0.85cb†	22.20d†	2.40a†	18.50cde†	54.70a†
CA 1012(2)	1.15a	25.75b	2.05bc	18.90bcd	50.95ef
EPSM 1667(3)	0.80c	22.45d	2.40a	19.20cb	53.50ab
EPSM 1667(4)	1.20a	26.20ab	2.05bc	18.20de	51.60cde
CA 2266(5)	1.10a	25.45b	2.05bc	19.30bc	50.40ef
CA 2266(6)	0.95b	23.60c	2.00bc	19.20bc	52.50bcd
CA 1044(7)	1.15a	25.70b	2.25ab	19.65b	50.25f
CA 1044(8)	0.80c	22.35d	1.65d	19.60b	54.65a
EPSM 1224(9)	1.15a	27.15a	2.00bc	17.85e	51.30def
Paymaster 145(10)	0.75c	22.45d	1.80cd	21.00a	52.90bc
FiberMax 958(11)	0.95b	25.65b	1.90cd	19.85b	50.80ef
Mean	0.98	24.45	2.05	19.20	52.14

†Mean values in the same column followed by the same letter are not significantly different at the 5% level of probability.

Table 7. Fatty acid data for 11 genotypes grown in Lubbock, 2000.

Genotype	%Saturated	%Unsaturated	U/S Ratio‡	O/L Ratio§
CA 1012(1)	25.45cd†	73.45a†	2.89a†	0.339f†
CA 1012(2)	28.95b	70.15d	2.43cd	0.371bcd
EPSM 1667(3)	25.65cd	73.00ab	2.85ab	0.359def
EPSM 1667(4)	29.45ab	69.90d	2.38cd	0.353def
CA 2266(5)	28.60b	70.00d	2.45cd	0.383abc
CA 2266(6)	26.55c	71.90bc	2.71b	0.366cde
CA 1044(7)	29.10ab	70.10d	2.41cd	0.391ab
CA 1044(8)	24.80d	74.40a	3.00a	0.359def
EPSM 1224(9)	30.30a	69.35d	2.29d	0.348ef
Paymaster 145(10)	25.00d	74.05a	2.96a	0.397a
FiberMax 958(11)	28.50b	70.85cd	2.49c	0.391ab
Mean	27.48	71.55	2.62	0.369

† Mean values in the same column followed by the same letter are not significantly different at the 5% level of probability.
‡ The ratio of percent unsaturated to saturated fatty acids.
§ The ratio of percent oleic to linoleic fatty acid.

Table 8. The fatty acid profiles of lipids extracted from cottonseed expressed as percent of total fatty acid of the 11 genotypes grown in Lubbock in 2001.

Genotype	14:0	16:0	18:0	18:1	18:2
CA 1012(1)	0.75b†	20.80e†	2.35a†	17.00f†	56.80a†
CA 1012(2)	1.00a	23.05bcd	2.35a	18.05e	53.25cde
EPSM 1667(3)	0.75b	21.00e	2.40a	18.15de	55.20b
EPSM 1667(4)	1.00a	24.50ab	2.35a	17.10f	52.55def
CA 2266(5)	0.95ab	23.35abc	2.30a	18.90ab	52.35def
CA 2266(6)	0.85ab	21.45de	2.30a	18.40cde	54.20bc
CA 1044(7)	0.75b	23.50ab	2.45a	18.65bc	52.05ef
CA 1044(8)	0.85ab	21.80cde	2.40a	19.20a	53.90bcd
EPSM 1224(9)	1.00a	24.90a	2.55a	17.25f	51.85ef
Paymaster 145(10)	0.75b	21.00e	2.35a	19.00ab	53.75bcd
FiberMax 958(11)	1.00a	24.40ab	2.55a	18.55bcd	51.20f
Mean	0.87	22.70	2.39	18.20	53.37

† Mean values in the same column followed by the same letter are not significantly different at the 5% level of probability.

Table 9. Fatty acid data for 11 genotypes grown in Lubbock, 2001.

Genotype	%Saturated	%Unsaturated	U/S Ratio‡	O/L Ratio§
CA 1012(1)	23.90d†	73.90a†	3.10a†	0.299d†
CA 1012(2)	26.40bc	71.40bc	2.71bcd	0.339bc
EPSM 1667(3)	24.15d	73.45a	3.05a	0.329c
EPSM 1667(4)	27.85ab	69.75de	2.51de	0.326c
CA 2266(5)	26.60bc	71.40bc	2.69cde	0.362a
CA 2266(6)	24.60d	72.65ab	2.96ab	0.340bc
CA 1044(7)	26.70abc	70.80cd	2.65de	0.359a
CA 1044(8)	25.05cd	73.20a	2.94abc	0.356a
EPSM 1224(9)	28.45a	69.20e	2.43e	0.333c
Paymaster 145(10)	24.10d	72.85ab	3.03a	0.354ab
FiberMax 958(11)	27.95ab	69.90de	2.50de	0.363a
Mean	25.97	71.68	2.77	0.342

† Mean values in the same column followed by the same letter are not significantly different at the 5% level of probability.
‡ The ratio of percent unsaturated to saturated fatty acids.
§ The ratio of percent oleic to linoleic fatty acid.

Correlations

Correlations were performed on each of the fatty acid profiles to determine if there was a relationship between the profile and cold tolerance. The Lubbock and New Deal emergence rate indices were based on the mean of four replications. The fatty acid profiles were based on the mean of two replications. Only the O/L ratio appeared to be related to cold tolerance. The O/L ratio was significantly (P=0.0808) correlated to the average ERI's in Lubbock. The O/L ratio in both 2000 and 2001 was significantly correlated to the Lubbock emergence rate index at the alpha level 0.1 in 2000 and at the alpha level 0.05 in 2001. The oleic profile in 2001 was significantly correlated to the Lubbock emergence rate index in 2001 at the alpha level 0.1. The linoleic profile in 2001 was significantly correlated to the Lubbock emergence rate index at the alpha level 0.1. The results are seen in Table 10.

Table 10. *P*-values for the average Lubbock emergence rate index in 2001 compared to the average fatty acid profile in 2000.

Fatty Acid	Pr>F
16:0 (palmitic)	0.4815
18:0 (oleic)	0.2085
18:1 (linoleic)	0.2961
%Saturated	0.6441
%Unsaturated	0.7044
Unsaturated/ Saturated Ratio	0.6566
Oleic/ Linoleic Ratio (O/L)	0.0808

Evaluation of Fatty Acid Profiles

Variability was observed within each of the fatty acid profiles at Lubbock in both 2000 and 2001. Because the O/L ratio was the only profile that appeared to be related to cold tolerance, the variation in this O/L ratio would be of most interest for future research. Cultivars from the Nutrition, Russian, and Mutant studies that have a low O/L ratio could be evaluated in future research for cold tolerance. This variability within the fatty acid profiles is listed in Tables 4.11, 4.12, and 4.13.

Table 4.11: The Nutrition study variability in fatty acid profiles in 2000 and 2001 at Lubbock.

Fatty Acid	2000	2001
14:0	0.8 to 1.4	0.7 to 1.1
16:0	21.2 to 29.0	19.6 to 26.3
18:0	1.8 to 2.9	2.0 to 2.8

18:1	16.4 to 22.2	14.1 to 19.7
18:2	43.6 to 53.3	50.8 to 58.7
18:3	0.0 to 0.5	0.0 to 0.3
O/L Ratio	0.317 to 0.455	0.246 to 0.365

Table 4.12: The Russian study variability in fatty acid profiles in 2000 and 2001 at Lubbock.

Fatty Acid	**2000**	**2001**
14:0	0.7 to 1.1	0.7 to 1.2
16:0	21.3 to 27.1	19.6 to 25.8
18:0	2.1 to 2.7	1.7 to 2.6
18:1	14.2 to 22.1	14.5 to 20.6
18:2	46.3 to 55.6	50.8 to 58.9
18:3	0.1 to 0.5	0.1 to 0.3
O/L Ratio	0.262 to 0.465	0.246 to 0.406

Table 4.13: The Mutant study variability in fatty acid profiles in 2000 and 2001 at Lubbock.

Fatty Acid	**2000**	**2001**
14:0	0.8 to 1.3	0.7 to 1.1
16:0	22.5 to 27.6	20.4 to 25.6
18:0	1.7 to 2.3	2.2 to 2.7
18:1	17.0 to 21.9	16.3 to 19.0
18:2	48.2 to 54.7	50.9 to 55.9
18:3	0.1 to 0.3	0.1 to 0.1
O/L Ratio	0.318 to 0.454	0.301 to 0.371

Summary

It is very difficult to define or measure cold tolerance. It is also difficult to definitely determine physiological factors that affect cold tolerance. This research shows that fatty acids may be related. Specifically, the Oleic/Linoleic ratio, which measures a shift within the unsaturated portion of the O/L ratio, is negatively correlated to cold tolerance. Nevertheless, it is not known whether the oleic and linoleic acids are directly related to cold tolerance. In previous work, Borth (1997) found that an increase in linoleic acid was a factor in seed maturity. These current findings may also be related to seed maturity. Future research is needed to determine if the O/L ratio could be a tool for selection in a breeding program for cold tolerance. Cultivars containing low oleic and high linoleic profiles resulting in lower O/L ratios should be screened and evaluated for their performance in the field under cold temperatures.

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