

THE INVESTIGATION OF COLD TOLERANCE IN COTTONSEED (*GOSSYPIMUM HIRSUTUM* L.) GERMINATION

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(Received 28th Jul 2018; accepted 26th Sep 2018)

Abstract. This study was carried out to determine the factors affecting the cold tolerance of cotton genotypes and to determine the relationships between cold tolerance and some seed characteristics in 2017. Fourteen cotton varieties belonging to *G. hirsutum* L. were used as material. Cold (15 °C) and normal (30 °C) germination condition parameters and fatty acid composition of the cotton varieties were investigated. Resulting, genetic structure and environmental conditions were effective in terms of fatty acid composition and cold tolerance parameters in addition to need of 21 days which is the time to germinate in cold conditions. Varieties Phantom and Stoneville-453 were the highest mid-tolerant, followed by BA-119, Sayar-314 and Deltapine-499, second degree mid-tolerant, and Berke, Deltaopal, SureGrow-125 and Deltapine-332 were medium tolerant in the third grade while Carmen, Gloria, Flora and Teks varieties were sensitive. Positive relationship was observed between cold tolerance and linolenic acid, stearic acid, saturated fatty acids, saturated/unsaturated fatty acid ratio, 100 seed weight, and seed density while negative relationship was between cold tolerance and oleic acid, linoleic acid, palmitic acid, unsaturated fatty acids, unsaturated/saturated fatty acid ratio, oleic/linolenic acid ratio, oleic/palmitic acid ratio, and linoleic/palmitic acid.

Keywords: cotton, chilly, stress, fatty acids, correlation

Introduction

Many ecological factors are involved in the growth and development of cotton plants. The main factors of these are temperature (high and low temperature), humidity, drought, wind and salinity. Germination in cotton seeds begins with water intake and continues with many biochemical and physiological changes such as softening of seed husk, seed swelling and cracking of seed husk by the effect of water (Khan, 2003). Temperature and humidity are very important factors in the germination of cottonseed. If the temperature is insufficient, germination cannot occur and cotton germination is known to be inhibited at low temperatures (Haigh and Barlow, 1997). The optimum germination temperature in cotton seeds is 30-34 °C (Lauterbach et al., 1999; Cole and Wheeler, 1974). For many cotton varieties, the physiological level of zero for germination is 15 °C (Marani and Dag, 1962; Wanjura and Buxton, 1972; Speed et al., 1996). If temperature falls below 15 °C during germination or if the cotton plant remains at temperatures of 10 °C or lower for several hours, long-term irreparable damage to cotton seeds can occur. The most important quality criteria in the seed are high viability and seed vigor (resistance to stress conditions, germination rate, etc.) (McDonald, 1999). Low temperatures cause irregularities and delays in germination and emergence of cotton seeds, resulting in the inability to obtain the desired plant populations in the field.

According to data, from 1970-2016 averaged for many years in Turkey from May to September period (hot period), soil temperature decreases from 5 cm to 10 cm soil depth while in other periods (cold/cool periods) it increases (*Fig. 1*). Cotton is grown between April 15 and May 15 in Diyarbakır/Turkey ecological conditions as a main

crop. The most important factor affecting the sowing date is the temperature of the air and the soil. According to long years average (1970-2016), temperature changes at 5 cm soil depth were 8 °C, 14 °C, 20.5 °C (MGM data) in March, April and May, respectively (Figs. 1 and 2). In addition, especially in April and May, low night temperatures in addition to rainfall causing low temperatures in this period are great disadvantages for cotton seeds germination and emergence.

One of the biggest obstacles to early planting and possibility to extent vegetation duration is the problem of germination and emergence at low temperatures. Especially after midnight, there are serious decreases in air temperatures. This affects cotton germination, emergence and seedling growth negatively. The second (re) planting should be done with the low temperature period that occurs after early planting and the lack of sufficient plant population, as well as with stand deficiencies and poor seedling growth in field conditions. The ability of cotton seeds to germinate at low temperatures will ensure that cotton sowing occurs earlier. This will lead to cotton vegetation time to be longer as well as positive development for cotton fiber yield (Sharma et al., 1998; Norfleet et al., 1998; Porter et al., 1997; Tomar et al., 1991; Raju and Kharache, 1994) and quality characteristics (Porter et al., 1997). Also, it will prevent the adverse effects of cotton development due to the low temperatures that occurs after the date of planting in the spring.

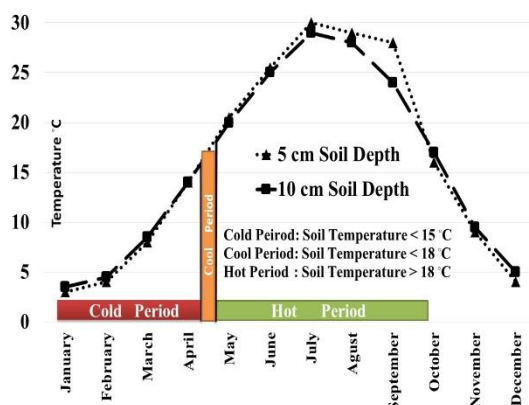


Figure 1. Temperature change averaged for many years (1970-2016) at 5 cm and 10 cm soil depth (MGM data)

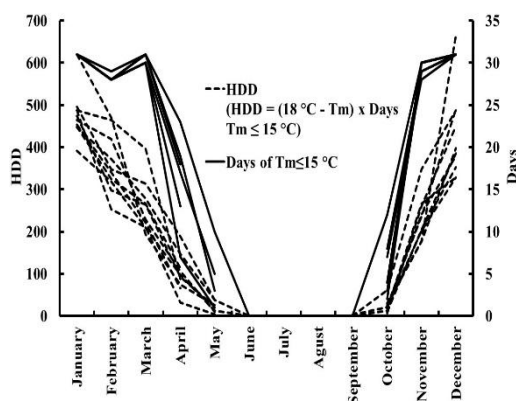


Figure 2. Change of HDD and $T < 15\text{ °C}$ days between 2008-2017 by months

Germination tests on cold conditions are also a stress test. When seed is subjected to cold stress conditions, this way, its performance against cold stress is examined. Many tests and parameters related to cold tolerance have been explored and used. Germination percentage, germination coefficient, speed of germination index, cold warm vigor index, high cold stress index, vigor index and germination index are the primaries. The cold- warm vigor index developed by Hopper is a very good cold tolerance test (Hopper et al., 1994; Metzger, 1987; Kerby et al., 1989).

Cotton fatty acid composition which is not always constant can vary respect to species, variety and environmental factors (Genter et al., 1957; Zimmerman et al., 1959; Ramey, 1984; Baydar and Turgut, 1999; Karaca and Aytac, 2012). Temperatures of 15 °C or higher causes phase transition at fatty acid composition of cottonseed and membrane lipids. When the temperature is below 15 °C, the oil crystallizes and the cell membrane loses its fluidity and selective permeability (Hall, 2003). Fatty acid composition in cottonseeds is manifested by low membrane function at low temperatures.

This study was carried out to determine the factors affecting the germination of cotton seeds in cold conditions and to determine the relationship between cold germination tolerance and some seed characteristics of cotton.

Materials and methods

The field study was carried out in the research area of Faculty of Agriculture at Dicle University, in the year of 2016 with 14 cotton (*G. hirsutum* L.) varieties (Teks, Stoneville-453, Stoneville-468, Deltaopal, Deltapine-499, Deltapine-332, BA-119, Carmen, Sayar-314, SureGrow-125, Fantom, Berke, Flora and Gloria).

The experimental area is located (37°890075 N, 40°275785 E) at 670 m above sea level. This region generally is characterized by a semiarid continental climate. The soil temperature change of 5 and 10 cm for long-term (1970-2016) have been presented in *Figure 1*. Change of day degree units (HDD) and $T < 15$ °C days period 2008-2017 by months have been presented in *Figure 2*. The average relative humidity is about 29.8% and total rainfall is 495 mm. The soil is low in organic matter and phosphorus. It has adequate calcium, potassium, and high clay content (48% to 68%) in the 0 to 160 cm profile. The sowing of cotton seeds was made by machine on 5 May (2016). All plots were treated with 20-20-0 (N-P-K) composite fertilizer to provide 75 kg N ha⁻¹ and 75 kg P₂O₅ ha⁻¹. All plots were furrow irrigated regularly. A total of 9 irrigations were applied. Plots were harvested twice by hand. Yield of four rows of plot were weighed and calculated for seed cotton yield. First harvest was done on 20 October (2016); second harvest was done on 12 November (2016).

The germination tests at 15 °C and 30 °C temperatures were carried out in the test laboratories of the Science and Technology Research and Application Center at Dicle University in 2017 with 4 replications according to randomized blocks design. Fatty acid composition of cotton seeds was analyzed with gas chromatography (GC).

Germination tests in cold (CC_{15C}) and normal (standard) conditions (NC_{30C}) were carried out in a temperature controlled growth cabinet having 80-100% humidity. Fifty seeds with 3 replications were placed in a wet paper towels and placed into growth cabinet. Counts were made on days 4, 7, 14 and 21 in both germination tests. At each count, a moist towel was opened and healthy developing seedlings having hypocotyl length of 3.8 cm or more was counted and recorded. The counted seedlings were

removed then the towel rewound and left in the growth cabinet again (Edmisten, 2000; Smith and Varvil, 1984; Tolliver et al., 1997). The parameters of germination coefficient (GC), speed of germination index (SGI), cold warm vigor index (CWVI) and high cold stress index (HCSI) were calculated with the following equations in cold and normal germination conditions.

GC can be calculated as *Equation 1* (Maguire, 1962):

$$GC = \frac{\sum G}{\sum GT} \times 100 \quad (\text{Eq.1})$$

SGI was calculated as *Equation 2* (AOSA, 1983, 1990):

$$SGI = \sum \frac{G_n}{T_n} \quad (\text{Eq.2})$$

where, G represents the number of germinated seeds counted on T day (4, 7, 14 and 21 days).

CWVI was calculated as *Equation 3*:

$$CWVI = GP_{NC7} + GP_{CC21} \quad (\text{Eq.3})$$

GP represents germinated percentage, NC represents normal condition (30 °C) at 7 day, CC represents cold condition (15 °C) at 21 day. The obtained CWVI value is considered to be excellent over 160, good between 140 and 159, moderate between 139 and 120 and weak if below 120 (Stauss and Hopper, 1983; Baughman et al., 1994, 2011).

HCSI was determined as *Equation 4* (Fischer and Maurer, 1978):

$$HCSI = \frac{\frac{G_{GPNC14} - G_{GPCC14}}{M_{GPNC14} - M_{GPCC14}}}{M_{GPNC14}} \quad (\text{Eq.4})$$

where, G: Genotype; M: Means; GPNC14: normal condition (30 °C) germination percent at 14 day; GPCC14: cold condition (15 °C) germination percent at 14 day. HCSI < 0.50 highly cold stress tolerant, 0.50 < HCSI < 1.00 moderately cold stress tolerant and HCSI > 1.00 susceptible.

The values obtained for each parameter were analyzed using JMP 7.0 (Copyright © 2007 SAS Institute Inc.) statistical package program. Means were grouped according to the LSD (0.05) test.

Results

Ratios for oleic acid (C18:1) (%), linoleic acid (C18:2) (%), linolenic acid (C18:3) (%), palmitic acid (C16:0) (%), stearic acid (C18:0) (%), saturated fatty acids (%), unsaturated fatty acids (%), unsaturated/saturated fatty acids, saturated/unsaturated fatty acids, oleic/linoleic acid, oleic/linolenic acid, linoleic/palmitic acid and means for 100

seed weight (g) and seed density (g.ml⁻¹) were given in *Tables 1, 2, and 3*. In terms of all the traits examined, varieties were statistically (1%) different than each other.

The ranges and the mean values for oleic acid 14.75-21.90% (18.70%), linoleic acid 36.99-48.95% (44.01%), linolenic acid 0.26-0.50% (0.37%), palmitic acid 20.75-27.75% (24.21%), stearic acid 2.05-3.58% (2.80%), saturated fatty acids 23.76-30.84% (27.00%), unsaturated fatty acids 54.37-70.47% (63.10%), unsaturated/saturated fatty acids ratio 2.05-2.69 (2.35), saturated/unsaturated fatty acids ratio 0.37-0.49 (0.43), oleic/linoleic acid ratio 0.31-0.50 (0.43), oleic/linolenic acid ratio 29.12-78.99 (52.73), oleic/palmitic acid ratio 0.58-1.00 (0.78), linoleic/palmitic acid ratio 1.61-2.06 (1.83) 100 seed weight 9.96-13.30 g (11.34 g), and seed density 1.01-1.15 g.ml⁻¹ (1.06 g.ml⁻¹) were measured (*Tables 1, 2 and 3*).

In terms of oleic acid trait, Carmen and Teks varieties; in terms of linoleic acid trait, Carmen, Flora, and Gloria varieties; in terms of linolenic acid and stearic acid traits, Fantom, and Stoneville-453 varieties; in terms of palmitic acid trait, BA-119, Berke, and SureGrow-125 varieties were the group in which the highest values were obtained (*Table 1*).

Table 1. Percentage of oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), palmitic acid (C16:0) and stearic acid (C18:0) of cotton varieties

Varieties	(C18:1)(%)	(C18:2)(%)	(C18:3) (%)	(C16:0) (%)	(C18:0)(%)
BA-119	19.57 e	44.57 d	0.38 de	27.67 a	2.94 cd
Berke	20.51 bcd	46.32 cd	0.41 bc	27.75 a	3.09 bc
Carmen	21.24 ab	48.95 a	0.26 i	24.17 de	2.10 h
Deltaopal	20.11 cde	40.39 f	0.30 h	25.09 bcd	2.20 gh
Deltapine-332	19.90 de	46.95 bc	0.26 i	24.25 de	2.05 h
Deltapine-499	20.77 bc	42.79 e	0.40 cd	20.75 g	3.01 c
Fantom	14.75 h	39.51 f	0.50 a	21.26 fg	3.58 a
Flora	14.99 h	47.92 abc	0.34 f	24.65 cd	2.68 ef
Gloria	17.74 f	48.20 ab	0.39 cde	26.03 bc	3.08 bc
Sayar-314	18.23 f	41.21 ef	0.31 gh	22.74 ef	2.34 g
Stoneville-453	16.88 g	36.99 g	0.49 a	21.04 g	3.45 a
Stoneville-468	19.87 de	42.34 e	0.37 e	24.26 de	2.80 de
SureGrow-125	15.35 h	45.06 d	0.43 b	26.35 ab	3.20 b
Teks	21.90 a	44.97 d	0.33 fg	22.80 ef	2.62 f
Means	18.70	44.01	0.37	24.21	2.80
CV (%)	3.14	2.79	4.26	4.45	4.03
Min-Max.	14.75-21.90	36.99-48.95	0.26-0.50	20.75-27.75	2.05-3.58
LSD _{0.05}	0.84	1.76	0.02	1.54	0.16
Prob.	**	**	**	**	**

(C18:1): oleic acid, (C18:2): linoleic acid, (C18:3): linolenic acid, (C16:0): palmitic acid, (C18:0): stearic acid, CV: coefficient of variance, LSD_{0.05}: least significant difference at 5% level, Prob: probability, * and ** significant at the 5% and 1% probability levels, respectively, means in each column followed by the same letter are not significantly different (p < 0.05)

In terms of saturated fatty acids trait, BA-119, Berke, SureGrow-125 varieties; in terms of unsaturated fatty acids trait, Carmen variety; in terms of unsaturated/saturated fatty acids ratio trait, Carmen, Deltapine-332, Deltapine-499, and Teks varieties; in terms of saturated/unsaturated fatty acids ratio trait, BA-119, Berke, and SureGrow-125 varieties; in terms of oleic/linoleic acids ratio trait, Deltaopal, Deltapine-499, and Teks varieties were the group in which the highest values were obtained (*Table 2*).

Table 2. Percentage of saturated fatty acid, unsaturated fatty acid, and ratio of unsaturated/saturated fatty acids, saturated/unsaturated fatty acids, oleic (C18:1)/linoleic acid (C18:2) of cotton varieties

Varieties	Sat. fatty acids (%)	Unsat. fatty acids (%)	Unsat./sat. fatty acids rate	Sat./unsat. fatty acids rate	(C18:1)/(C18:2) rate
BA-119	30.61 ab	64.53 cd	2.11 de	0.47 ab	0.43 de
Berke	30.84 a	67.25 b	2.18 cde	0.45 abc	0.44 de
Carmen	26.28 cd	70.47 a	2.68 a	0.37 e	0.43 de
Deltaopal	27.30 c	60.81 fg	2.23 cd	0.44 bc	0.50 a
Deltapine-332	26.31 cd	67.12 b	2.55 a	0.39 e	0.42 e
Deltapine-499	23.76 f	63.97 de	2.69 a	0.37 e	0.48 ab
Fantom	24.85 def	54.77 h	2.20 cde	0.45 bc	0.37 f
Flora	27.33 c	63.26 de	2.31 bc	0.43 cd	0.31 h
Gloria	29.12 b	66.34 bc	2.27 bc	0.43 cd	0.36 f
Sayar-314	25.09 def	59.75 g	2.39 b	0.41 d	0.44 de
Stoneville-453	24.50 ef	54.37 h	2.22 cd	0.45 bc	0.45 cd
Stoneville-468	27.06 c	62.59 ef	2.31 bc	0.43 cd	0.46 bc
SureGrow-125	29.56 ab	60.85 fg	2.05 e	0.49 a	0.34 g
Teks	25.42 de	67.21 b	2.64 a	0.37 e	0.48 ab
Means	27.00	63.10	2.35	0.43	0.43
CV (%)	4.00	2.13	4.52	4.50	4.26
Min-Max.	23.76-30.85	54.37-70.47	2.05-2.69	0.37-0.49	0.31-0.50
LSD _{0.05}	1.55	1.93	0.15	0.03	0.03
Prob.	**	**	**	**	**

(C18:1): oleic acid, (C18:2): linoleic acid, CV: coefficient of variance, LSD_{0.05}: least significant difference at 5% level, Prob: probability, * and ** significant at the 5% and 1% probability levels, respectively, means in each column followed by the same letter are not significantly different ($p < 0.05$)

In terms of oleic/linoleic rate trait, Carmen, and Deltapine -332 varieties; in terms of oleic/palmitic rate trait, Deltapine-499, and Teks varieties; in terms of linoleic/palmitic rate trait, Carmen, Deltapine-332, Deltapine-499, Flora, and Teks varieties; in terms of 100 seed weight and seed density traits, Fantom, and Stoneville-453 varieties were the group in which the highest values were obtained (*Table 3*).

The average values of germination coefficients (GC₁₅ and GC₃₀) (determined by *Eq. 1*), speed of germination index (SGI₁₅ and SGI₃₀) (determined by *Eq. 2*), cold-warm

vigor index (CWVI) (determined by *Eq. 3*) and high cold stress index (HCSI) (determined by *Eq. 4*) were given in *Table 4* for cold and normal conditions of cotton constituting the material.

Table 3. Ratio of oleic (C18:1)/linolenic (C18:3), oleic (C18:1)/palmitic (C16:0), linoleic (C18:2)/palmitic (C16:0), means of 100 seed weight and seed density of cotton varieties

Varieties	(C18:1)/(C18:3) rate	(C18:1)/(C16:0) rate	(C18:2)/(C16:0) rate	100 seed weight (g)	Seed density (g.ml ⁻¹)
BA-119	50.81 d	0.70 de	1.61 g	10.96 cd	1.04 c
Berke	49.69 de	0.73 d	1.67 fg	11.42 bc	1.06 bc
Carmen	78.99 a	0.88 b	2.02 a	12.03 b	1.02 d
Deltaopal	67.22 b	0.80 c	1.61 g	10.45 de	1.08 b
Deltapine-332	75.77 a	0.82 c	1.94 abc	10.18 e	1.02 d
Deltapine-499	51.77 d	1.00 a	2.06 a	11.98 b	1.06 bc
Fantom	29.12 h	0.69 de	1.86 bcd	13.3 a	1.13 a
Flora	43.64 f	0.60 e	1.94 abc	10.89 cd	1.02 d
Gloria	45.22 ef	0.68 f	1.85 bcd	10.98 cd	1.01 d
Sayar-314	57.86 c	0.80 c	1.82 cde	11.45 bc	1.07 b
Stoneville-453	33.99 g	0.80 c	1.76 def	13.01 a	1.15 a
Stoneville-468	53.05 d	0.81 c	1.74 defg	10.57 de	1.07 bc
SureGrow-125	35.42 g	0.58 f	1.70 efg	11.48 bc	1.08 b
Teks	65.72 b	0.96 a	1.97 ab	9.96 e	1.02 d
Means	52.73	0.78	1.83	11.34	1.06
CV (%)	5.94	4.98	5.36	4.28	1.55
Min-Max.	29.12-78.99	0.58-1.00	1.61-2.06	9.96-13.30	1.01-1.15
LSD _{0.05}	4.48	0.06	0.14	0.69	0.02
Prob.	**	**	**	**	**

(C18:1): oleic acid, (C18:2): linoleic acid, (C18:3): linolenic acid, (C16:0): palmitic acid, CV: coefficient of variance, LSD_{0.05}: least significant difference at 5% level, Prob: probability, * and ** significant at the 5% and 1% probability levels, respectively, means in each column followed by the same letter are not significantly different ($p < 0.05$)

In terms of all the traits examined, there were statistical difference of 1% between genotypes. Ranges and mean values for germination coefficient in the cold conditions (GC₁₅), germination coefficient (GC₃₀) under normal conditions, speed of germination index (SGI₁₅) in cold condition, speed of germination index (SGI₃₀) under normal condition, CWVI and HCSI were 5.65-6.23% (5.99%), 11.20-15.03% (13.64%), 1.62-2.45 (1.88%), 7.47-9.06% (8.16%), 110.41-152.45 (130.17), 0.86-1.13 (1.00) (*Table 4*).

In terms of CWVI trait, Fantom, Sayar-314 and Stoneville-453 varieties; in terms of HCSI trait, Flora, Gloria, and Teks varieties; were the group in which the highest values were obtained (*Table 4*).

Table 4. Percent of GC (15 °C and 30 °C), SGI (15 °C and 30 °C), CWVI and HCSI of cotton varieties

Varieties	GC 15 °C	GC 30 °C	SGI 15 °C	SGI 30 °C	CWVI	HCSI
BA-119	6.17 ab	14.58 ab	1.86 c	7.47 f	134.10 cd	0.95 g
Berke	6.23 a	14.81 ab	1.89 c	8.98 a	138.45 bc	1.00 ef
Carmen	5.82 de	14.33 a-d	1.82 cd	8.69 ab	114.66 e	1.06 bcd
Deltaopal	6.08 bc	13.86 cde	1.72 de	8.19 bd	127.40 d	1.00 ef
Deltapine -332	5.78 ef	11.51 f	1.69 e	7.61 ef	128.12 d	1.04 cde
Deltapine-499	6.13 ab	15.03 a	1.94 c	8.12 ce	134.54 cd	0.97 fg
Fantom	6.21 ab	13.49 e	2.2 b	7.60 ef	152.45 a	0.86 h
Flora	5.65 f	11.51 f	1.64 e	7.76 df	112.82 e	1.10 ab
Gloria	5.66 f	11.20 f	1.62 e	7.55 f	110.41 e	1.08 abc
Sayar-314	6.12 ab	14.52 abc	2.09 b	8.33 bc	145.35 ab	0.93 g
Stoneville-453	6.16 ab	14.69 ab	2.45 a	9.06 a	150.65 a	0.87 h
Stoneville-468	6.13 ab	13.63 de	1.85 c	7.78 df	128.03 d	0.94 g
SureGrow-125	5.96 cd	14.31 bcd	1.84 cd	8.43 bc	129.56 d	1.02 de
Teks	5.68 f	13.43 e	1.64 e	8.59 ac	115.81 e	1.13 a
Means	5.99	13.64	1.88	8.16	130.17	1.00
CV (%)	1.69	3.67	4.75	4.58	4.25	3.48
Range	5.65-6.23	11.20-15.03	1.62-2.45	7.47-9.06	110.41-152.45	0.86-1.13
LSD _{0.05}	0.15	0.72	0.13	0.53	7.91	0.05
Prob.	**	**	**	**	**	**

GC: germination coefficient, SGI: speed of germination index, CWVI: cold warm vigor index, HCSI: high cold stress index, °C: centigrade degree, CV: coefficient of variance, LSD_{0.05}: least significant difference at 5% level, Prob: probability, * and ** significant at the 5% and 1% probability levels, respectively, means in each column followed by the same letter are not significantly different ($p < 0.05$)

Discussion

When the germination rate at 30 °C, considered as the normal germination temperature for cotton plant, was examined, it was determined that the maximum germination occurred on the 7th day, whereas the germination count at 21 day determined to be the maximum germination time at 15 °C, which was considered as the cold condition (Figs. 3 and 4). This suggests that low temperature causes delayed of germination time and a significant reduction of germination rate.

Results of the germination coefficient (GC₁₅), speed of germination index (SGI₁₅), cold-warm vigor index (CWVI) and high cold stress index (HCSI) values that are indicative for cold tolerance were parallel to each other. The high values of the GC₁₅, SGI₁₅ and CWVI were confirmed by the low HCSS. Our findings had similarity with Tuck et al. (2006).

It is desirable that the CWVI values are high and the HCSI value is low for tolerance to cold. No HCSI value < 0.50 was obtained from the varieties forming the material, and so no cold-tolerant variety was detected. Since $0.50 < \text{HCSI} < 1.00$ was detected in

Stoneville-453, Fantom, Sayar-314, BA-119, Stoneville-468 and Deltapine-499 varieties, they were called as mid-tolerant but other varieties were susceptible to cold. However, Fantom (0.86) and Stoneville-453 (0.87) varieties were found to be the highest mid-tolerant varieties with the lowest HCSI values (Table 4; Fig. 8). BA-119, Sayar-314 and Deltapine-499 varieties showed themselves as medium tolerant varieties at the second level (Fig. 8).

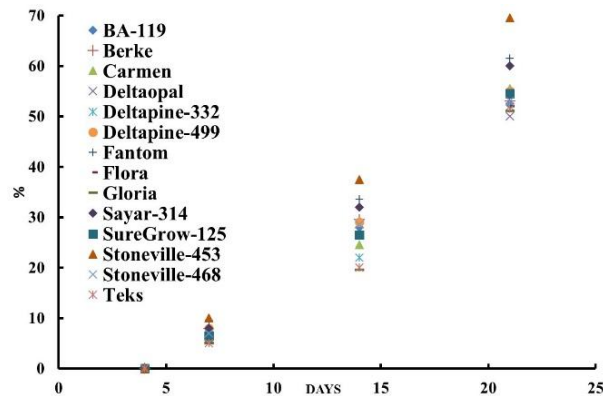


Figure 3. Germination percentage at cold (15 °C) conditions on days 4, 7, 14 and 21

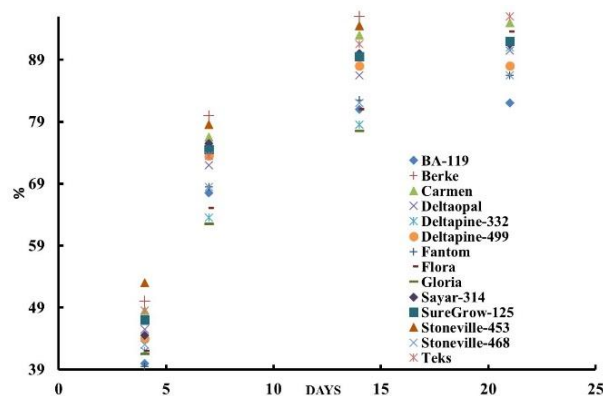


Figure 4. Germination percentage at standard (30 °C) conditions on days 4, 7, 14 and 21

Values of CWVI > 160 were not determined, while values of Fantom, Stoneville-453 and Sayar-314 ranged from 140 to 160 that were indicated as *good*. On the other hand, Stoneville-468, BA-119, Deltapine-499, Berke, Deltaopal, SureGrow-125 and Deltapine-332 varieties were found to be *medium* because CWVI values were between 120-140 while Carmen, Gloria, Flora and Teks varieties were *weak* because of values were smaller than 120 (Table 4; Fig. 8). Similar results have been obtained with varieties in terms of CWVI and HCSI characteristics.

Among the cotton varieties used were Fantom and Stoneville-453, the highest mid-tolerant, BA-119, Sayar-314 and Deltapine-499 varieties, secondly medium tolerant, Berke, Deltaopal, SureGrow-125 and Deltapine-332 medium tolerant with 3th grade, Carmen, Gloria, Flora and Teks varieties were thought to be sensitive to cold. Our findings are similar to the results of Bartonkowski et al. (1978), Maluf and Tifchelaar (1982), Hailstones and Smith (1988), Speed et al. (1996), Borth (1997), Al-Bahrain and

Khary (2000), Sheikh et al. (2002), Down et al. (2010), Özbek (2011), Karaca and Aytac (2012), Kaya (2014), Kazmi et al. (2015), Bolek et al. (2016), while those of Dogras et al. (1977) and Lukonge et al. (2007) are different. This difference may be due to the difference in genotype or environmental conditions.

The cold-tolerant genotype in cotton seeds is characterized by high CWVI and low HCSI. It is seen that Fantom and Stoneville-453 varieties where the highest values for linolenic acid, stearic acid, 100 seed weight and seed density traits are obtained are also the highest varieties in which CWVI values are obtained (*Fig. 8*). This suggests that linolenic acids, stearic acids, 100 seed weight and seed density are important properties for CWVI (*Figs. 11 and 13*). The positive correlation between linolenic ($r = +0.54^{**}$), stearic acids ($r = +0.43^{**}$), 100 seed weight ($r = +0.52^{**}$) and seed density ($r = +0.77^{**}$) with the CWVI supports this situation (*Table 5*). In this case, the amount of high linolenic acids, stearic acids, 100 seed weight and seed density cottonseed, is more tolerant to high cold stress, reveals the result. This suggests that linolenic acids, stearic acids, 100 seed weight and seed density are important properties for CWVI and HCSI (*Figs. 6, 7, 11 and 13*). However, significant positive correlation was found between cold tolerance in the cottonseed and saturated fatty acids ($r = +0.22$), saturated/unsaturated ($r = +0.32$), but this was not statistically significant (*Table 5; Figs. 5–14*).

Table 5. Pearson correlation coefficients to examine the relationship of GC (15 °C and 30 °C), SGI (15 °C and 30 °C), CWVI and HCSI

Traits	GC ₁₅	GC ₃₀	SGI ₁₅	SGI ₃₀	CWVI	HCSI
Oleic acid	-0.01	+0.27*	-0.31*	+0.27	-0.23	+0.30*
Linoleic acid	-0.66**	-0.46**	-0.69**	-0.16	-0.71**	+0.74**
Linolenic acid	+0.45**	+0.28*	+0.59**	+0.08	+0.54**	-0.55**
Palmitic acid	-0.09	-0.12	-0.48**	-0.09	-0.31*	+0.34*
Stearic acid	+0.35**	+0.2	+0.48**	+0.02	+0.43**	-0.44**
Sat. fatty acids	+0.02	+0.08	+0.39**	+0.09	+0.22	-0.25
Unsat. fatty acids	-0.51**	-0.21	-0.69**	+0.02	-0.66	+0.72
Uns./sat. ratio	-0.36**	-0.07	-0.19	+0.09	-0.32	+0.34
Sat./uns. ratio	+0.38**	+0.11	+0.19	+0.07	+0.32	-0.33
Oleic/linoleic	+0.38**	+0.52**	+0.12	+0.35**	-0.20	+0.16
Oleic/linolenic	-0.30*	-0.08	-0.46**	-0.09	-0.42**	+0.45**
Oleic/palmitic	-0.06	+0.32*	-0.05	+0.28*	-0.01	+0.05
Linoleic/palmitic	-0.46**	-0.25	-0.11	-0.06	-0.3*	+0.29*
100 seed weight	+0.45**	+0.39**	+0.72**	+0.21	+0.52**	-0.58**
Seed density	+0.71**	+0.5**	+0.75**	+0.27*	+0.77**	-0.75**

GC: germination coefficient, SGI: speed of germination index, CWVI: cold warm vigor index, HCSI: high cold stress index, °C: centigrade degree, * and ** significant at the 5% and 1% probability levels, respectively

On the other hand, our findings, oleic acid, linoleic acid, palmitic acid, unsaturated fatty acids, unsaturated/saturated, oleic/linoleic, oleic/linolenic, oleic/palmitic, and linoleic/palmitic should be low for high cold tolerance in the cottonseed (Figs. 9, 10, 12 and 14). Correlations between CWVI and oleic ($r = -0.23$), linoleic ($r = -0.71^{**}$) and palmitic ($r = -0.31^{*}$) acids, unsaturated fatty acids ($r = -0.66$), unsaturated/saturated ($r = -0.32$), oleic/linoleic ($r = -0.20$), oleic/linolenic ($r = -0.42^{**}$), oleic/palmitic ($r = -0.01$), and linoleic/palmitic ($r = -0.30^{*}$) support this (Table 5). All results obtained for CWVI are also supported by HSCI results. The existence of a positive relationship ($r = +0.78^{**}$) between HCSI and CWVI confirms this similarity (Table 5). Our findings are similar to those of Judith and Christiansen (1976), Carmichael (2007), Özbek (2011), but differ from the findings of Edmisten (2000).

100 seed weight and seed density traits can vary significantly depending on the conditions of growing the cotton plant. As 100 seed weight and seed density increases in favorable growing conditions, it decreases in unfavorable conditions. It is also an indicator of the maturity of the cotton seed. While mature cotton is high in seed density, low value is obtained in immature cotton seeds. This suggests that cold tolerance is influenced not only by the genetic structure of the cotton seed, but also by the environmental conditions and seed maturity of the cotton seed. This reveals the fact that the conditions for growing cotton seeds are of great importance for cold tolerance. Our findings showed similarities with the findings of Bartee and Krieg (1974), Tripathi and Khan (1990), Bolek (2006, 2010) and Pahlavani et al. (2008).

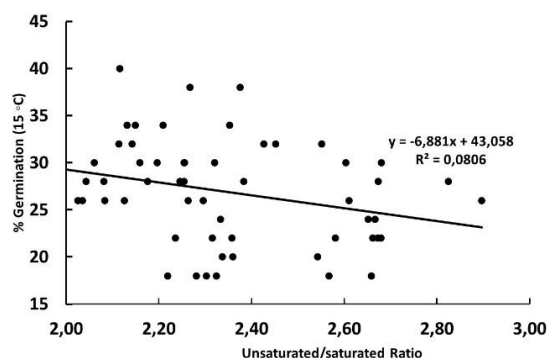


Figure 5. Relationship between germination percentage and unsat./sat. fatty acids ratio at cold (15 °C)

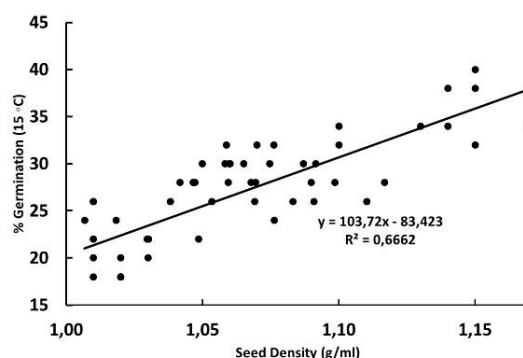


Figure 6. Relationship between germination percentage and seed density at cold (15 °C)

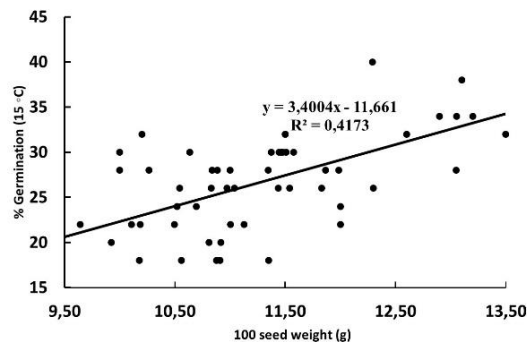


Figure 7. Relationship between germination percentage and 100 seed weight at cold (15 °C)

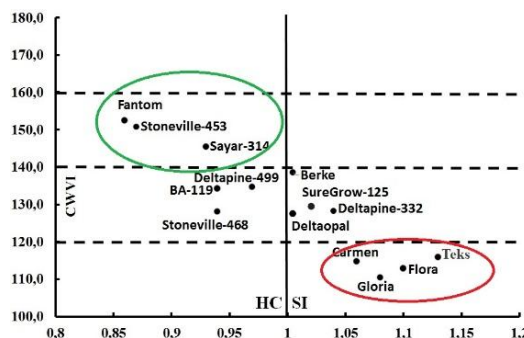


Figure 8. Relationship between CWVI and HCSI at cold (15 °C)

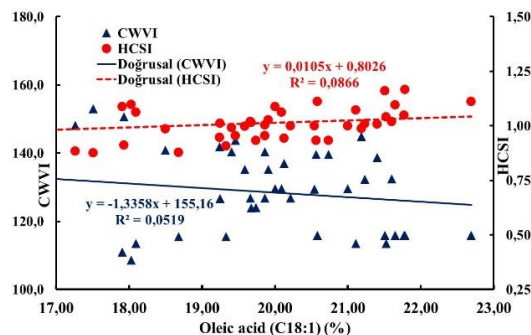


Figure 9. Relationship between CWVI and C18:1 at cold (15 °C)

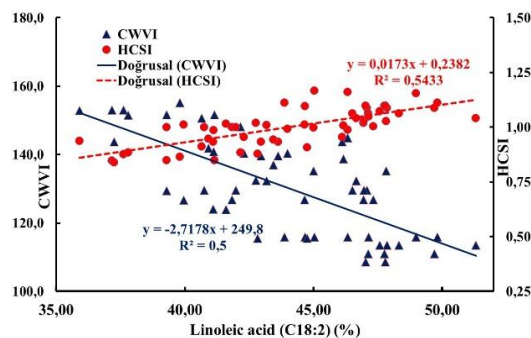


Figure 10. Relationship between CWVI and C18:2 at cold (15 °C)

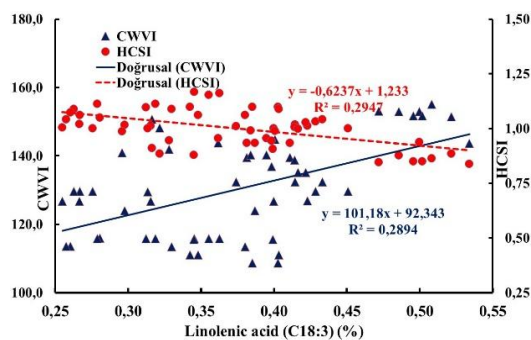


Figure 11. Relationship between CWVI and C18:3 at cold (15 °C)

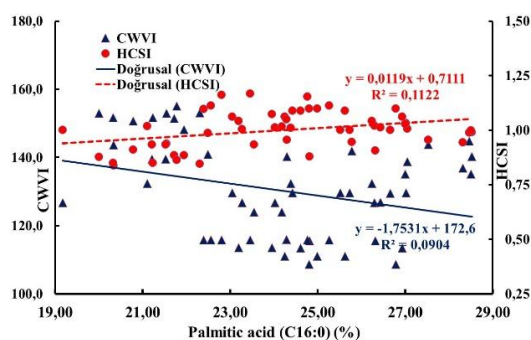


Figure 12. Relationship CWVI and C16:0 at cold (15 °C)

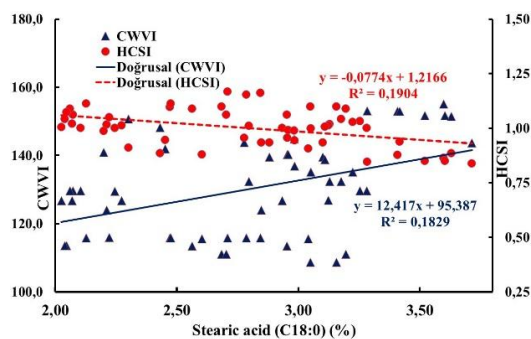


Figure 13. Relationship between CWVI and C18:0 acid at cold (15 °C)

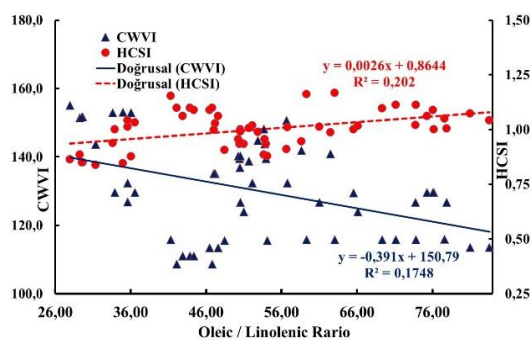


Figure 14. Relationship between CWVI and oleic/linolenic acid ratio at cold (15 °C)

Conclusion

Genetic structure and environmental conditions are very effective factors in terms of fatty acid composition and cold tolerance parameters. The cold condition for germination of cotton seed is 15 °C that prolongs the duration up to 21 days which brings with many negativity. There were not cold tolerant variety used in this experiment but cultivars could be ordered as Fantom and Stoneville-453 were the highest medium-tolerant, BA-119, Sayar-314 and Deltapine-499 were the second medium-tolerant, Berke, Deltaopal, SureGrow-125 and Deltapine-332 varieties were medium-tolerant at the third level, while Carmen, Gloria, Flora and Teks varieties were sensitive. In future studies, it is recommended to use Stoneville-453 and Fantom cotton varieties as highest medium-tolerant genotypes and Carmen, Gloria, Flora and Teks cotton varieties as sensitive genotypes. It is recommended that the properties of linolenic acid, stearic acid, saturated fatty acids, saturated/unsaturated fatty acids ratio, 100 seed weight and seed density are high in cotton seeds to reduce cold stress during germination. The CWVI and HCSI features are ideal indicators for cold tolerance.

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