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Relationship between Fusarium Wilt Disease and Fatty Acids Content of Cottonseed

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ABSTRACT

Ten genotypes of cotton were evaluated for resistance to Fusarium wilt cause by *Fusarium oxysporum* f. sp. *vasinfectum*. The genotypes were divided into two groups based on their reactions to the disease. The first group included five susceptible genotypes (9/2017, 32/2017, 58/2017, 63/2017, and 68/2017) and the second group includes the other five resistant genotypes (123/2017,131/2017, 133/2017, 135/2017, and 137/2017). Diseases incidence in the first group ranged from 83.33% to 100%, while it ranged in the second group from 0% to 20%. Cottonseeds of genotypes contained twenty-two distinct fatty acids. Eight fatty acids (archidic, palmitic, stearic, linoleic, linolenic, myrsitic, oleic, and vaccinic) were found in different amounts in all the tested genotypes. Five fatty acids (capric, caprylic, heptadecenoic, pentadeceonic, and tetradecenoic) were found only in the resistant genotypes. Percentage of saturated acids in cottonseed genotypes was 31.719%, while unsaturated acid was 67.001%. Positive significant correlation was found between Fusarium wilt incidence and each of arachidic acid and behenic acid. Negative significant correlation was found between disease incidence and each of heptadecenoic and pentadecenoic acid. Cluster analysis showed that fatty acid profile was unable to differentiate among cotton genotypes based on their reaction to Fusarium wilt. However, fatty acid profiles differentiated among some genotypes regardless of their reactions to Fusarium wilt disease. Therefore, fatty acids could be used in seed purity tests.

Keywords: Fusarium wilt; Fatty acids; Cotton seed.

INTRODUCTION

Cotton is the most important strategic crop in Egypt and plays a major role in the Egyptian national economy, because it is an important source of raw material in cotton textile industry as well as being a source for the production of cotton seed oil and used in animal feeding. Fusarium wilt is one of the most economically damaging cotton disease. The first reported of Fusarium wilt in Egypt was in 1902 (Fahmy, 1927). It caused by the fungal pathogen *Fusarium oxysporum* f. sp. *vasinfectum* (Atk.) Sayd. & Hans. (FOV).

In addition to a worldwide distribution, Fusarium wilt occurs in all domesticate cotton species {*Gossypium arborium, G. barbadense*, *G. herbeceum*, and *G. hirsutum* (Armstrong and Armstrong, 1960)}. It causes yellowing, wilting defoliation, vascular tissue damage and ultimately death (Wang *et al*., 2009). Once a field is infested with *F. oxysporum* f. sp. *vasinfectum*, the fungus usually persists indefinitely (Smith and Snyder, 1975; and Wood and Ebbels, 1972). Usage of resistant cultivars is the most effective and practical method for controlling the disease (Doan and Davis, 2014).

Fatty acids, ubiquitous in nature, play a crucial role in life processes. They are involved in cell energy storage, membrane structure, and various signaling pathways. In addition, fatty acids in animal fat, plant oil, and other sources are an important part of human nutrition (Liu *et al*., 2008).

A number of fatty acids have been shown to inhibit or stimulate the growth and sporulation of pathogenic fungi in plants (Aly *et al*., 2011). Harman *et al*., (1980) found that palmitic acid stimulated germination of condiospores of *Fusarium solani* f. sp. *pisi* while stearic and linolenic acids were ineffective. The antifungal activities of nine fatty acids (butyric, caproic, caprylic, capric, lauric, myristic, palmitic, oleic, and linoleic) against four phytopathogenic fungi: *Alternaria solani*, *Colletotrichum lagenarium*, *Fusarium oxysporum* f. sp. *cucumerium* and *F. oxysporum* f. sp. *lycopersici*, were assessed by measuring mycelial growth and spore germination via petri dish assay (Liu *et al*., 2008). Except for oleic acid, the fatty acids tested were observed to inhibit mycelial growth and spore germination of one or more of the tested fungi.

Several biochemical and molecular methods have been used to evaluate resistance of cotton cultivars to Fusarium wilt such as protein electrophoresis, Random Amplified Pollymorphic DNA (RAPD), Simple Sequence Repeats (SSR), Inter Simple Sequence Repeats (ISSR), Sequence Related Amplified Polymorphism (SRAP) (Aly *et al*., 2007; Zhang *et al*., 2002; Zhang *et al*., 2005; Frelichowski *et al*., 2006; and He *et al*., 2007). On the other hand, very few attempts have been made to use fatty acid profile of cottonseed as a potential method for differentiation between resistant and susceptible genotypes. Fatty acids had been shown to be correlated with resistance or susceptibility to plant diseases. For example, *Pinus sylvestris* seed containing more unsaturated fatty acids and a large amount of erucic acid, were more resistant to damping of fungi (Garzywacz and Rosochackon 1977). The oleic and linolenic acid content in cotyledons of cotton were correlated with Fusarium wilt resistance, with significant differences in fatty acid content existing between wilt-resistant and susceptible varieties. Aly *et al*., (2004) used fatty acid composition of linseed to distinguish between powdery mildew resistant and susceptible genotypes.

Therefore, the objectives of the present study were (1) to evaluate fatty acids of cottonseed as a potential method for differentiation between cotton genotypes based on their reaction to Fusarium wilt and (2) to evaluate fatty acids as a potential method to differentiate between cotton genotypes regardless of their reaction to Fusarium wilt as this method could be useful in seed purity tests.

MATERIALS AND METHODS

Cotton genotypes:

The ten cotton genotypes (*G. barbadense* L.) used in this study were supplied by Cotton Breeding Section, Cotton Research Institute. These genotypes were 9/2017, 32/2017, 58/2017, 63/2017. 68/2017, 123/2017, 131/2017, 133/2017, 135/2017, and 137/2017.

Evaluation of genotypes against Fusarium wilt under greenhouse conditions:

This test was conducted in the greenhouse of cotton and Fiber Crops Disease Research Section in 2018. The fungal inoculum was a mixture of equal parts (W/W) of 50

isolates of *Fusarium oxysporum* f. sp. *vasinfectum* (race 3), which obtained from fungal collection of cotton pathology section, Plant Pathology Research Institute (PPRI) Giza, Egypt. 500ml glass bottles, each contained 50 gm of sorghum grains and 40 ml of tap water were autoclaved and then infested with the fungus inocula, taken from one-week old culture on potatoes dextrose agar medium (PDA). After three weeks, autoclaved clay loam soil was infested with a mixture of the isolates at rate 10g/kg soil. Infested soil was dispensed in 10 cm diameter clay pots with three replicates for each genotype. The pots planted with ten seeds per pot. Greenhouse temperature ranged from 28°C to 35°C.

Evaluation of Fusarium wilt incidence:

After 45 days from planting date disease incidence was recorded by counting the percentage of all seedlings, which showed external or internal symptoms (vascular discoloration) (Abd-Elsalam *et al*., 2009).

Extraction of oil from seeds:

Extraction of fatty acids and analysis were conducted at the Regional Center of Food and Feed Stuff, Agriculture Research Center, Giza, Egypt. To characterize the fatty acid composition of cottonseed genotypes each sample was dried at 60°C and grind in a coffee grinder until the whole seeds were crushed. Oil was extracted by using Soxtec apparatus (FOSS Tecator, Auckland, NZ). Fatty acids in the extracted oil were performed by method of AOAC (2016).

Fatty acid composition of cotton seed genotypes:

Fatty acid composition of samples were trans esterified into their corresponding fatty acid methyl esters (FAMEs) using methanolic sodium hydroxid and boron triflouride with methanol as described by the AOAC (2016). The FAMEs were quantified by Shematizu Gas Chrematograph (GC) Series 2010 equipped with a 2010+S (auto sampler) (Japan) and interfaced with a Flame Ionization Detectors (FID). The GC was equipped with a temperature programmable column. The column phase was Supplco DB Wax (Carbowax) with the following dimensions: 30m long, 0.25 mm i.e. with a 0.25 µm phase thickness. Helium was used as a carrier gas with flow rate of 45ml/min. One µL was injected using the inlet in a split mode. The head pressure was set at 2 psi and the split vent flow was 7mL/m. The injector temperature was 250°C. The column flow rate at 2 psi was 0.68 mL/m. The column temperature was maintained at 200°C for 10°C/S and was held at 26°C for 80 min. The detector was operated in the selected ion monitoring mode. Fatty acids were identified by retention times obtained from the FAME standards (Sigma Company, St. Louis, MO).

Statistical analysis:

Data were subjected to analysis of variance (ANOVA) and least significant difference (LSD) were calculated to compare between genotypes means by using MSTAT-C statistical package. Pearson correlation coefficient (r) was calculated to measure the degree of association between fatty acid profile of each pair of cotton genotypes. Based on these data, a correlation matrix was constructed and from this matrix genotypes were clustered by the unweighted pair group method based on arithmetic mean (UPGMA). Cluster analysis was performed using SPSS 6.0 software package.

RESULTS AND DISCUSSION

Regarding Table 1, cotton genotypes can be divided into two groups. The first group included the highly susceptible genotypes (9/2017, 32/2017, 58/2017, 63/2017, and 68/2017) where Fusarium wilt incidence ranged from 83.33% to 100%. The second group included the highly resistance lines (123/2017, 131/2017, 133/2017, 135/2017, and 137/2017) where the disease incidence ranged from 0% to 20%. The differences between any two genotypes from the two groups were always significant while the differences between genotypes within each group was non-significant; however, the difference between genotypes 123/2017 and 137/2017 in the resistant group was a notable exception.

Table 1. Reaction of selected cotton genotypes to Fusarium wilt under greenhouse conditions

No.	Cotton genotypes	Fusarium wilt incidence (%)
1	9/2017	83.33[a]
2	32/2017	100.00
3	58/2017	90.00
4	63/2017	96.67
5	68/2017	96.67
6	123/2017	20.00
7	131/2017	3.33
8	133/2017	3.33
9	135/2017	3.33
10	137/2017	0.00

LSD (P≤ 0.05) = 17.86 [a] **Mean of three replicates**

Qualitative profiles of fatty acids extracted from the ten cotton genotypes are shown in Table 2. Twenty two fatty acids were detected (ten saturated fatty acids and twelve unsaturated). Eight fatty acids were found in all the tested cotton genotypes. These fatty acids were arachidic, linoleic, linolenic, myristic, oleic, palmitic, stearic, and vaccinic. Capric and caprlyic acids were found in the two highly resistant genotypes only (63/2017 and 68/2017).

Table 2. Qualitative profiles of fatty acids extracted from ten cotton genotypes

Fatty acids	Genotypes									
	9	32	58	63	68	123	131	133	135	137
Arachidonic	+[a]	-[b]	-	-	+	+	-	-	-	-
Behenic	+	+	+	+	+	+	+	+	+	-
Capric	-	-	-	-	-	-	+	+	-	-
Caprlyic	-	-	-	-	-	-	+	+	-	-
Eicosaenoic	+	-	+	+	-	-	-	-	+	-
Erucic	+	+	+	+	+	+	-	+	+	-
Gadolic	-	-	+	+	+	+	+	+	+	-
Heptadecanoic	-	-	-	-	-	-	+	+	+	+
Hexadecatrienoic	-	+	+	+	+	-	+	-	+	-
Lauric	-	-	+	+	+	+	+	+	-	-
Linoleic	+	+	+	+	+	+	+	+	+	+
Linolenic	+	+	+	+	+	+	+	+	+	+
Myristic	+	+	+	+	+	+	+	+	+	+
Oleic	+	+	+	+	+	+	+	+	+	+
Palmitic	+	+	+	+	+	+	+	+	+	+
Palmitioleic	+	+	+	+	+	+	-	+	+	+
Palmitolic	+	-	-	-	+	+	+	-	-	-
Pentadecanoic	-	-	-	-	-	-	+	+	+	-
Stearic	+	+	+	+	+	+	+	+	+	+
Tetradecanoic	-	-	-	-	-	-	+	+	-	-
Vaccinic	+	+	+	+	+	+	+	+	+	+
Non Identified F.A.	+	+	+	+	+	+	+	+	+	+

[a] **(+) Fatty acid is present** [b] **(-) Fatty acid is absent**

Quantitative profiles of fatty acids (Table 3) showed that the fatty acids which found in all tested cotton genotypes were different in quantities in different genotypes. Hamza *et al*., (1988) observed varied values for fatty acid-composition

in Egyptian cotton genotypes. Dowd *et al*., (2010) studied fatty acid profile in twenty cotton genotypes at two different locations, and recorded significant differences among genotypes. Lukonge *et al*., (2007) evaluated twenty-four upland cotton genotypes for fatty acid profile and noted significant differences among genotypes.

Table 3. Quantitative (%) profiles of fatty acids extracted from ten cotton genotypes

Fatty acids	Genotypes									
	9	32	58	63	68	123	131	133	135	137
Arachidic	0.34	0.45	0.55	0.46	0.37	0.23	0.33	0.35	0.32	0.33
Arachidonic	0.14	0.00	0.00	0.00	0.44	0.35	0.00	0.00	0.00	0.00
Behenic	0.11	0.16	0.34	0.17	0.19	0.10	0.10	0.15	0.11	0.00
Capric	0.00	0.00	0.00	0.00	0.00	0.00	0.54	0.38	0.00	0.00
Caprlyic	0.00	0.00	0.00	0.00	0.00	0.00	0.18	0.16	0.00	0.00
Eicosaenoic	0.65	0.00	0.48	0.11	0.00	0.00	0.00	0.00	0.39	0.00
Erucic	0.11	0.11	10.09	2.13	0.16	0.19	0.00	0.24	0.14	0.00
Gadolic	0.00	0.00	2.34	0.55	0.17	0.21	0.11	0.12	0.24	0.00
Heptadecenoic	0.00	0.00	0.00	0.00	0.00	0.00	0.38	0.41	0.11	0.11
Hexa decatrienoic	0.00	0.23	0.17	0.19	0.13	0.00	0.10	0.00	0.16	0.00
Lauric	0.00	0.00	0.14	0.11	0.14	0.13	0.73	0.48	0.00	0.00
Linoleic	37.93	47.54	38.56	41.81	30.25	27.86	38.20	41.80	36.00	43.60
Linolenic	0.18	0.13	2.83	0.68	0.26	0.27	0.40	0.31	0.12	0.21
Myristic	0.79	0.64	0.74	0.73	0.93	0.93	3.03	2.21	0.73	0.63
Oleic	27.88	20.13	17.62	21.39	30.93	32.97	20.95	20.20	24.10	24.20
Palmitic	24.69	25.26	21.04	26.74	26.36	26.27	26.00	25.10	27.16	25.10
Palmitioleic	1.25	0.73	0.69	0.71	1.81	2.17	0.00	0.85	0.13	0.15
Palmitolic	0.19	0.00	0.00	0.00	0.31	0.31	0.10	0.00	0.00	0.00
Pentadecenoic	0.00	0.00	0.00	0.00	0.00	0.00	0.57	0.28	0.43	0.00
Stearic	4.27	3.32	2.68	3.11	5.90	5.46	4.94	3.83	4.58	3.58
Tetradecenoic	0.00	0.00	0.00	0.00	0.00	0.00	0.19	0.14	0.00	0.00
Vaccinic	1.11	0.66	0.94	0.78	1.37	1.52	1.26	0.96	1.00	0.90
Non Identified F.A.	0.36	0.64	0.79	0.33	0.28	1.03	1.89	2.03	4.28	1.19

Fatty acid composition (%) of cottonseeds (Table 4) showed twenty two fatty acids. Linoleic acid showed that highest percentage of cottonseed fatty acids (38.355%), while tetradecenoic acid showed the lowest percentage (0.033%). Cottonseed composed of ten saturated fatty acids represent 31.719% of fatty acids and twelve unsaturated fatty acids represent 67.001% of fatty acids. This result is in agreement with Kazmi *et al*., (2015) who found that fatty acid profile of cottonseed showed that it consists of 70% unsaturated acids and 30% saturated acids. Oleic acid and linoleic acid collectively make most of unsaturated fatty acid (93.12%). Kazmi *et al*., (2015) stated that oleic and linoleic acids, collectively make the unsaturated fatty acids.

Table 4. Fatty acid composition of cotton seed

Fatty acids	Formula	Content (%)
Arachidic	C20:0	0.373[a]
Arachidonic	C20:4ω6	0.093
Behenic	C22:0	0.143
Capric	C10:0	0.092
Caprlyic	C8:0	0.034
Eicosaenoic	C20:1ω7	0.163
Erucic	C22:1ω9	1.317
Gadolic	C20:1ω9	0.374
Heptadecanoic	C17:0	0.101
Hexadecatrienoic	C16:3ω4	0.098
Lauric	C12:0	0.173
Linoleic	C18:2ω6	38.355
Linolenic	C18:3ω3	0.539
Myristic	C14:0	1.136
Oleic	C18:1ω9	24.037
Palmitic	C16:0	25.372
Palmitioleic	C16:1ω7	0.849
Palmitolic	C16:1ω9	0.091
Pentadecanoic	C15:0	0.128
Stearic	C18:0	4.167
Tetradecanoic	C14:1ω5	0.033
Vaccinic	C18:1ω7	1.052
Non Identified F.A.	-	1.282

LSD (P≤ 0.05) = 1.677

[a] Mean of ten genotypes as shown in Table (3).

Correlation between fatty acids (x) of cottonseed and Fusarium wilt incidence (y) are shown in Table (5). There was positive significant correlation between arachidic acid and Fusarium wilt incidence (r=0.68, p≤0.03). Another positive significant correlation was found between behenic acid and disease incidence (r=0.623, p≤0.05). On the other hand, a negative significant correlation was found between heptadecenoic acid and Fusarium wilt incidence (r= -0.697, p≤0.025) similarly another negative significant correlation was found between pentadecenoic acid and disease incidence (r=-0.652, p≤0.04). Aly *et al*., (2011) stated that the significant positive or negative (r) value does not necessarily prove that fatty acids are beneficial or detrimental to fungi. Thus, the primary utility of correlation analysis was to identify the potentially interactive pairs of fatty acids and fungi. The positive (r) value may indicate that fatty acids had stimulatory effects on the growth and sporulation of cottonseed fungi. On the other hand negative (r) value could be attributed to inhibitory activities of fatty acids (Aly *et al*., 2011).

The dendogram shown in Fig. 1 divided the cotton genotypes qualitatively into six groups. The first group included the two susceptible genotypes 58/2017 and 63/2017 in addition to the resistant genotype 135/2017. The second group included susceptible genotype 32/2017. Group three included genotypes 9/2017, 68/2017 (susceptible), and 123/2017 (resistant). The resistant genotype 137/2017 was placed in a separate group remotely related to the other genotypes. The resistant genotypes 131/2017 and 133/2017 were placed in a single group unrelated to the other genotypes.

Fatty acids profiles could be used to differentiate qualitatively among resistant genotype 133/2017 and each of susceptible genotypes 58/2017 and 63/2017 in seed purity regardless of their reaction to Fusarium wilt.

Table 5. Correlation between fatty acids (x) of cotton seed and Fusarium wilt incidence (y)

Fatty acids	Linear correlation coefficient (r)	Probability Level
Arachidic	0.680*[a]	0.030
Arachidonic	0.215	0.550
Behenic	0.623*	0.050
Capric	-0.515	0.128
Caprlyic	-0.524	0.120
Eicosaenoic	0.273	0.445
Erucic	0.380	0.279
Gadolic	0.337	0.341
Heptadecanoic	-0.697*	0.025
Hexadecatrienoic	0.563	0.090
Lauric	-0.417	0.230
Linoleic	0.099	0.786
Linolenic	0.331	0.350
Myristic	-0.496	0.145
Oleic	-0.039	0.916
Palmitic	-0.285	0.426
Palmitioleic	0.360	0.307
Palmitolic	0.130	0.721
Pentadecanoic	-0.652*	0.041
Stearic	-0.263	0.463
Tetradecanoic	-0.517	0.126
Vaccinic	-0.257	0.474
Non Identified F.A.	-0.713*	0.021

[a] **Linear correlation coefficient (r) which measures the degree of association between Fusarium wilt incidence and the designated fatty acid.**

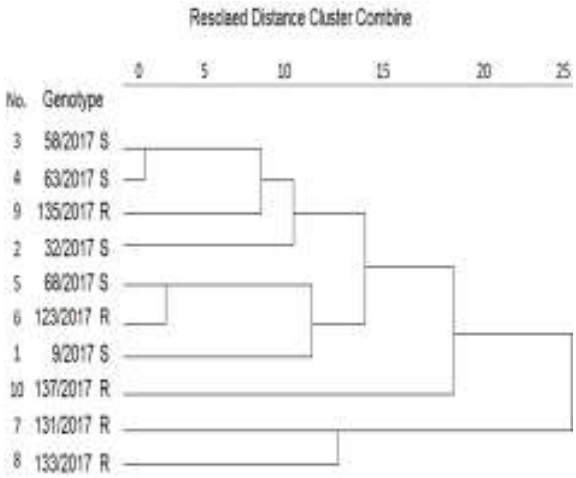

Fig. 1. A dendrogram based on average linked cluster analysis of qualitative profiles of fatty acids from cotton seeds. The reactions of the tested genotypes were resistant (R) or susceptible (S).

The dendrogram shown in Fig. 2 classified the cotton genotypes quantitatively into three groups. The first group included the susceptible genotype 68/2017 and resistant genotype 123/2017 (distance = 0.0). The second group at distance 5.1 include most of the genotypes regardless of their reactions to the disease. The third group included only the susceptible genotype 58/2017. Fatty acids profiles could be used to differentiate quantitatively between resistant genotype 123/2017 and all the other resistant genotypes in seed purity tests. Fatty acid profile was unable to differentiate among cotton genotypes based on their reaction to Fusarium wilt disease.

However, fatty acid profile could be used to differentiate among genotypes in seed purity tests regardless of their reaction to Fusarium wilt.

Our results were in agreement with Guo *et al*., (1991) who reported that the fatty acids in the seeds and root system showed no correlation with Fusarium wilt resistance.

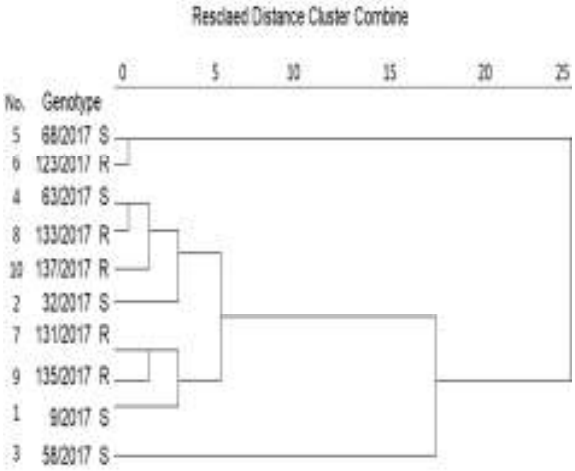

Fig. 2. A dendrogram based on average linked cluster analysis of quantitative profiles of fatty acids from cotton seeds. The reactions of the tested genotypes were resistant (R) or susceptible (S).

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العلاقة بين مرض ذبول الفيوزاريوم ومحتوى بذرة القطن من الأحماض الدهنية

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فى هذه الدراسة أختبرت عشرة تراكيب وراثية من القطن من حيث االمقاومة للاصابة بذبول الفيوزاريوم المتسبب عن فطر فيوزاريوم اوكسيسبورم طراز فازينفيكتم. انقسمت التراكيب الوراثية الى مجموعتين، المجموعة الأولى قابلة للاصابة بذبول الفيوزاريوم وهى التراكيب الوراثية ٢٠١٧/٩ و ٢٠١٧/٣٢ و ٢٠١٧/٥٨ و ٢٠١٧/٦٣ بالاضافة الى ٢٠١٧/٦٨. المجموعة الثانية اشتملت على التراكيب الوراثية المقاومة لذبول الفيوزاريوم وهى ٢٠١٧/١٢٣ و ٢٠١٧/١٣١ و ٢٠١٧/١٣٣ و ٢٠١٧/ ١٣٥و ٢٠١٧/١٣٧. تراوحت نسبة حدوث الاصابة فى مجموعة التراكيب الوراثية القابلة للاصابة من ٨٣,٣٣٪ الى ١٠٠٪ فى حين تراوحت نسبة حدوث الاصابة فى التراكيب الوراثية المقاومة من صفر ٪ الى ٢٠٪. عند تقدير الأحماض الدهنية فى التراكيب الوراثية أمكن الحصول على اثنين وعشرون حامض دهنى. قدرت الأحماض الدهنية كميا ونوعيا فى بذور التراكيب الوراثية بعد تحويلها الى مشتقات لاسترات الميثيل. وقد وجدت ثمانية أحماض دهنية فى جميع التراكيب الوراثية المختبرة (أركيدك – بالميتك – ستريك – لينوليك – لينولنيك – ميرستيك – أوليك – وفاكسينيك) ولكن بنسب مختلفة فى كل صنف. ظهرت بعض الأحماض الدهنية فى التراكيب الوراثية المقاومة فقط. هذه الأحماض هى كابريك وكابرليك وهيبتاديكانويك وبنتاديكانويك وتيترا ديكانويك. وقد أظهر تكوين الأحماض الدهنية فى بذور التراكيب الوراثية المختلفة أن حامض اللينوليك يمثل نسبة ٣٨,٣٥٥٪ من جميع الأحماض الأخرى فى حين ان حامض تيترا ديكانويك كان أقل الأحماض الدهنية تواجدا (٠,٠٣٣٪). كانت نسبة الأحماض الدهنية المشبعة هى ٣١,٧١٩٪، فى حين نسبة الأحماض الدهنية الغير مشبعة كانت ٦٧,٠٠١٪ عند دراسة الارتباط بين الأحماض الدهنية وحدوث الاصابة بالفيوزاريوم فى التراكيب الوراثية المختلفة، وجد أن هناك ارتباط موجب بين حدوث الاصابة وكل من حامض الاركيديك وحامض البيهينك، فى حين وجد ارتباط سالب بين حدوث الاصابة وبين كل من حامض الهيبتاديكانويك وحامض البنتاديكانويك. أظهر التحليل العنقودى عدم قدرة الأحماض الدهنية على التفرقة بين التراكيب الوراثية من حيث المقاومة أو القابلة للاصابة بذبول الفيوزاريوم، فى حين كانت قادرة على التفرقة بين بعض التراكيب الوراثية بغض النظر عن المقاومة أو القابلية للمرض مما يجعلها مفيدة للتفرقة بين التراكيب الوراثية فى اختبارات نقاوة البذرة.