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Assessment of correlation between powdery mildew severity and fatty acid profile of flaxseed oil

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An outdoor pot experiment was conducted in 2016/ 2017 growing season at Giza Agricultural Research Station to evaluate reactions of 15 flax cultivars to powdery mildew (PM) in 6/4/2017 and 20/4/2017. The tested cultivars showed a wide range disease severity (DS) ranged from 8.84% on Ottowa 770B to 78.35% on Giza10 in the first evaluation date and from 11.94 on Brasium to 86.72%on Giza 10 in the second evaluation date. Gas-liquid chromatography analysis of the fatty acid composition of cultivar seeds revealed that the unsaturated fatty acids Oleic, linoleic, and linolenic were predominant in oils of all the tested cultivars. The mean percentages of the three fatty acids were 35.69, 14.32, and 30.12%, respectively. Pearson correlation coefficient was calculated to measure the degree of association between PM severity (X) and the percentage of each fatty acids (Y). None of the fatty acids, expect $y_{14,}$ was significantly correlated with PM severity. This lack of correlation could be attributed to the fact that amounts of fatty acids are highly heritable characters, which indicates that they were mainly governed by genetic factors and therefore, slightly affected by biotic stress resulted from infection by PM. On the other hand, we do not have an immediate biological explanation for the positive and significant correlation between DS and the percentage of the fatty acid y_{14}. In spite of the significance of this correlation, it was not practically important due to the very low percentage of this fatty acid in the oil.

Keywords: fatty acid profile, powdery mildew, diseases resistance

INTRODUCTION

Flax (*Linum usitatissimum* L.) powdery mildew caused by *Oidium lini*, Skoric, *Oidium lini* has been reconsidered and is now known as a synonym of *Podosphaera lini* (Preston C.D.; Cook R.T.A. 2019, Braun et al., 2019). Powdery mildew (PM) is currently considered the most common conspicuous widespread and easily recognized foliar disease of flax in Egypt. Flax is grown for both seeds and fibers production in the lower Egypt particularly in the northern governorates. The flax growing areas is characterizing by the prevalence of warm, wet weather during the late period of flax producing season. Such weather favors epiphytotic spread of Powdery mildew (PM) when pathogenic isolates of the causal fungus occur. Over the last two decades, the importance of this disease has increased probably due to the appearance and rapid distribution of new races capably of attacking the previously resistant cultivars (Aly et al. 1994, Chauhan et al. 2017). Currently, resistant is not available in the commercially grown flax cultivars in Egypt but some imported flax cultivars such as Dakota are resistance to PM (Aly et al. 2002, Osman et al., 2018). Therefore, in years when environmental conditions favour the development of the disease, foliar application of fungicides has become the

only commercially available management practice for its management (Aly et al. 1994). Although the causal organism of PM on flax, in other countries has been reported as *Erysiphe pologoni* DC. EX Marat (Nyvall 1981), in Egypt, it has not been observed in its perfect stage. Therefore, in the current research the fungus will be referred to in its imperfect conidial stage, i.e. *Oidium lini* Skoric (Cullis et al. 2019). In recent years, there has been an increasing demand for domestic flax, leading to a need for increasing the growing area of flax in Egypt (A.A. Aly, personal observation).

Flaxseed contains 26- 45% oil (Kajla et al., 2014) approximately 22% of oil is located in the seed coat and 4% in the embryo. The oil is present mainly as triacylglycerols in oil bodies having an average diameter of 1.3µm (Daun et al. 2003). Approximately 70% of all flaxseed oil produced worldwide is destined for technical applications and 30% for food production (Kajla et al., 2014, Dzuvor et al., 2018).

Oleic, linoleic, and linolenic are considered to be unsaturated acids since they have double bonds between adjoin carbons. These double bonds are the active sites in the molecule where other elements or compounds may combine or react. Oleic has one double bond, linoleic has two double bonds, and linolenic three double bonds, Quality of flaxseed oil for industrial uses is determined by percentages of these three unsaturated fatty acids particularly linolenic acid (Kenaschuk, 1975). Oil quality is commonly measured as iodine value (Nykter *et al*., 2006).

Most of the known biological activities of flaxseeds have been also assigned to linoleinc acid (Oomah, 2001). Thus, the high linoleinc acid content of flaxseed oil makes it a valuable raw material for food and medicinal purposes. In the aliphatic chain of fatty acid, carboxyl end is called alpha and the methyl end is called omega.

From the omega and there is a double bond at third carbon called omega-3 and double bond of sixth carbon atom called omega- 6. Body of the human being cannot produce its own omega-3 and omega-6 fatty acids so there is a requirement of omega-3 and omega-6 rich food in the daily diet (Twining *et al.* 2017, Dinicolantonio *et al.* 2018).

Oil quantity of flaxseeds is expressed as seed oil percentage or oil yield / feddan (one feddan= 4200m^2) (Aly *et al.* 2001, Hussein and Omer, 2013) while oil quality is determined based on fatty acid composition (Nykter *et al*., 2006).

The correlation between PM intensity ratings (independent variables) and quantity of oil (dependent variables) was evaluated and the correlation was always non-significant (Aly et al., 2017; Hussein and Omer 2013). On the other hand, as far as we know, no attempts have been made to evaluate the degree of association between PM intensity ratings and fatty acid composition (quality) of flaxseed oil.

Therefore, the objectives of the present study were to determine PM severity (independent variable) on 15 flax cultivars and evaluate the correlation between the PM severity and fatty acid composition (dependent variable) of seeds obtained at harvest from these cultivars.

MATERIALS AND METHODS

2.1. The reaction of flax cultivars to powdery mildew

An outdoor pot experiment was conducted in 2016/2017 growing season at Giza Agricultural Research Station, to evaluate PM severity on 15 flax cultivars. These cultivars were randomly selected from the collection of flax germplasm available at Cotton and Fiber Crops Diseases Research Section, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt. Seeds of flax cultivars were planted on 15 November 2016 in natural soil dispensed in 50-cm diameter clay pots (20 seeds /pot). The pots were distributed in a randomized complete block design five replicates/blocks). PM was allowed to developed naturally, and disease severity (DS) was rated visually in 6/4 and 20/4/2017 based on the percentage of infected leaves /plant in a random sample of 10 plants /pot (Nutter et al.,1991). DS was not rated based on the percentage of infected leaf area because, at this stage of disease development, the surface of the infected leaf was almost completely covered with fungal growth.

2.2. Extraction of crude fat.

Random samples of seeds, taken at harvest from each cultivar, were used for extraction of crude fat by using dimethyl ether according to AOAC (2000).

2.3. Identification of fatty acids by using gas liquid chromatography (GLC).

The fatty acids of the oil were converted to methyl esters using sodium methoxide according to Ackman et al. (1973). Methyl esters fatty acid were identified by using Perkin-Elmer 8310 GC, with column 200 cm stainless steel 10% silar 10 °C on 100/120 Gas Chrom Q. Both of injector and flame ionization detector (FID) temperature were

150^0C and 250^0C, respectively. The carrier gas was nitrogen with 20 ml/min. flow rate. The program began with 100°C for 2min., then increased to 200 °C with a rate of 10/min and isothermally at 200 °C for 25 min. Fatty acid contents were calculated from chromatogram peak areas and expressed as a percentage (w/w) of the total fatty acids.

2.4. Statistical analysis.

The experimental design of the outdoor pot experiment was a randomized complete block with five replicates (blocks). Statistical analysis was performed with the software package SPSS 6.0. Analysis of variance (ANOVA)was used to compare means pf PM severity on the tested cultivars. ANOVA was also used to compare means of the separated fatty acids. the linear correlation coefficient was calculated to evaluate the degree of association between PM severity (x)and the percentage of each fatty acid (Y).

RESULTS

Environmental condition in 2016 /2017 growing season was favorable for epiphytotic spread of the disease. This was apparent as these environmental conditions resulted in 78.35 and 86.72% DS on Giza 10(Table 1), which is known as highly susceptible (A.A. ALY, personal observation). In general, most of the tested cultivars tended to be more susceptible in the second date. However, DS was highly correlated (r=0.766, p = 0.001) in the two evaluation dates. The tested cultivars showed a wide range of DS ranged from 8.84% on Ottowa 770 B to 78.35% on Giza10 in the first evaluation date and from 11.94% on Brasium to 86.72 on Giza10 in the second evaluation date.

The GLC analysis of fatty acids composition of flaxseed oil (Table 2) revealed that the unsaturated fatty acids oleic, linoleic, and linolenic were predominant in oils of all the tested cultivars. The mean percentages of the three fatty acids were 35.69, 14.32, and 30.12% respectively (Table 3). Pearson correlation coefficient was calculated to measure the degree of association between PM severity (X) and percentage of each fatty acid (Y). None of the fatty acids, expect Y14, was significantly correlated with PM severity (Table 4).

Table 1. Powdery mildew severity ratings on 15 flax cultivars in an outdoor pot experiment in Giza in 2016/2017 growing season.

Cultivar		Geographic	Powdery Mildew severity[a] in	
No	Name	origin	6/4/2017(X_1)	20/4/2017(X_2)
V1	Ottowa770B	U.S.A.	8.84	33.28
V2	Dakota	U.S.A.	17.80	45.12
V3	Bombay	U.S.A.	22.86	54.51
V4	Wilden	U.S.A.	31.43	61.64
V5	Cass	U.S.A.	35.76	59.00
V6	Marshall	U.S.A.	53.32	58.45
V7	Sakha1	Egypt	39.04	58.51
V8	Sakha2	Egypt	50.50	62.84
V9	Sakha3	Egypt	57.08	54.91
V10	Sakha4	Egypt	50.96	59.04
V11	Giza 9	Egypt	60.06	78.36
V12	Giza 10	Egypt	78.35	86.72
V13	Line 11	Egypt	61.51	63.61
V14	Electra	Belgium	68.11	59.77
V15	Brasium	Belgium	19.13	11.94
LSD (p ≤ 0.05)			7.30	6. 43

[a]Powdery mildew severity is the percentage of infected leaves/plant in a random sample of 10 plants /pot. Powdery mildew severity was highly correlated (r=0.766, p= 0.001) in the two dates

Table 2. Fatty acid composition (%, w/w of the total fatty acids of seeds of 15 Flax cultivars).

Fatty acid		Flax Cultivar[a]														
No	**Formula[b]**	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**	**V10**	**V11**	**V12**	**V13**	**V14**	**V15**
Y1	**C10:0**	0.13	0.95	0.00	0.11	0.72	0.55	0.22	0.36	0.55	0.73	0.00	0.22	0.22	0.00	0.32
Y2	**C12:0**	0.22	0.24	0.00	0.`16	0.29	0.98	0.30	0.29	0.39	0.00	0.20	0.31	0.28	0.00	1.24
Y3	**C14:0**	0.97	1.06	0.00	0.65	1.27	3.42	1.22	0.69	1.78	0.53	0.61	1.42	1.15	0.40	0.45
Y4	**C14:1Ω5**	0.00	0.00	0.00	0.00	0.14	0.38	0.15	0.00	0.20	0.00	0.00	0.15	0.00	0.00	0.00
Y5	**C15:0**	0.22	0.24	0.00	0.00	0.39	0.91	0.27	0.16	0.37	0.00	0.00	0.30	0.24	0.00	0.00
Y6	**C16:0**	8.47	9.76	7.86	8.19	9.33	15.15	10.17	9.41	10.60	7.86	11.30	9.60	8.79	6.83	7.10
Y7	**C16:1Ω7**	0.38	0.63	0.13	0.34	0.38	0.86	0.46	0.29	0.48	0.25	0.45	0.44	0.41	0.26	0.00
Y8	**C16:2Ω4**	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Y9	**C17:0**	0.19	0.21	0.19	0.00	0.31	0.86	0.32	0.14	0.26	0.00	0.56	0.39	0.24	0.00	0.00
Y10	**C16:3Ω4**	0.00	0.00	0.00	0.00	0.10	0.18	0.10	0.00	0.00	0.00	0.20	0.00	0.00	0.00	0.00
Y11	**C18:0**	7.18	7.52	6.70	5.27	6.65	7.99	7.13	6.27	7.24	6.30	7.41	6.58	7.23	5.41	6.10
Y12	**C18:1Ω9**	37.04	38.98	40.99	35.06	34.27	32.95	34.96	36.42	35.10	35.10	39.90	39.00	31.7	32.30	31.60
Y13	**C18:2Ω6**	9.73	10.21	13.87	12.25	12.42	15.87	17.99	23.80	15.30	15.30	10.30	10.00	15.00	16.80	15.90
Y14	**C18:2Ω4**	0.33	0.32	0.13	0.27	0.27	0.37	0.23	0.17	0.45	0.22	0.60	0.61	0.30	0.22	0.00
Y15	**C18:3Ω3**	34.26	28.83	28.65	37.02	32.54	18.27	25.50	21.16	26.10	32.50	27.10	29.70	33.50	36.90	36..70
Y16	**C18:4Ω3**	0.16	0.16	0.00	0.00	0.19	0.45	0.20	0.00	0.25	0.00	0.00	0.20	0.20	0.00	0.00
Y17	**C20:0**	0.25	0.30	0.42	0.24	0.27	0.32	0.36	0.44	0.32	0.33	0.35	0.32	0.30	0.24	0.25
Y18	**C20:1Ω9**	0.15	0.31	0.30	0.25	0.18	0.15	0.16	0.19	0.00	0.20	0.30	0.20	0.23	0.26	0.27
Y19	**C22:0**	0.25	0.27	0.33	0.19	0.27	0.18	0.24	0.20	0.20	0.23	0.41	0.21	0.20	0.20	0.20
Y20	**Unidentified**	0.07	0.01	0.43	0.00	0.01	0.03	0.02	0.01	0.39	0.45	0.92	0.35	0.00	0.18	0.00

[a]Names and geographic origins of flax cultivars are shown in Table 1. **[b]Names of fatty acids are shown in Table 3.**

Table 3: Fatty acid composition of flaxseed.

Fatty acid		Content[a] (% w/w of the total Fatty acids
Formula	**Name**	
C10:0	Capric acid	0.34
C12:0	Lauric acid	0.33
C14:0	Myristic acid	1.04
C14:1 Ω 5	Tetradecenoic acid	0.07
C15:0	Pentadecanoic acid	0.21
C16:0	Palmitic acid	9.36
C16: 1 Ω 7		0.38
C16:2 Ω 4		0.01
C17:0	Heptadecanoic acid	0.25
C16:3 Ω 4	Hexadecatrienoic acid	0.04
C18:0	Stearic acid	6.73
C18:1 Ω 9	Oleic acid	35.69
C18:2 Ω 6	Linoleic acid	14.32
C18:2 Ω 4		0.30
C18:3 Ω 3	Linolenic acid	30.12
C18:4 Ω 3	Alpha octadecatetraenic acid	0.12
C20:0	Arachidic acid	0.31
C20:1 Ω 9	Gadolic acid	0.21
C22:0	Behenic acid	0.24
Unidentified	Unidentified acids	0.19

[a]Mean of 15 cultivars. LSD (p≤ 0.05) = 19.81

Table 4: Correlation between powdery mildew severity (X) on 15 flax cultivars and Fatty acid composition (Y)of seeds obtained at harvest from these cultivars.

Fatty acid		Powdery Mildew severity[b]in	
No	**Formula[a]**	**6/4/2017(X_1)**	**20/4/2017(X_2)**
Y1	C10:0	-0.150[c](0.593)[d]	-0.177(0.528)
Y2	C12:0	-0.143 (0.611)	-0.506(0.054)
Y3	C14:0	0.257 (0.355)	-0.151(0.591)
Y4	C14:1 Ω 5	0.300 (0.278)	0.204(0.467)
Y5	C15:0	0.163 (0.563)	0.112(0.691)
Y6	C16:0	0.253 (0.364)	0.321(0.244)
Y7	C16:1 Ω 7	0.218 (0.436)	0.356(0.193)
Y8	C16:2 Ω 4	0.130 (0.644)	0.031(0.913)
Y9	C17:0	0.295 (0.286)	0.389(0.152)
Y10	C16:3 Ω 4	0.185 (0.508)	0.301(0.275)
Y11	C18:0	-0.036 (0.897)	0.048(0.864)
Y12	C18:1 Ω 9	-0.144 (0.609)	0.339(0.216)
Y13	C18:2 Ω 6	0.201 (0.473)	-0.072(0.799)
Y14	C18:2 Ω 4	0.539 (0.038)	0.683(0.005)
Y15	C18:3 Ω 3	0.283 (0.306)-	-0.358(0.190)
Y16	C18:4 Ω 3	0.157 (0.576)	0.100(0.723)
Y17	C20:0	0.162 (0.564)	0.361(0.186)
Y18	C20:1 Ω 9	0.207 (0.459)-	-0.027(0.923)
Y19	C22:0	0.171 (0.543)-	0.193(0.490)
Y20	Unidentified	0.374 (0.170)	0.453(0.090)

[a]Names of fatty acids are shown in Table 3
[b]Percentage of infected leaves/ plant in a random sample of 10 plants/pot.
[c]Pearson´s correlation coefficient, which measures the degree of association between Powdery mildew severity and the designated fatty acid
[d]Porbabilty level.

DISCUSSION

In the present study, GLC was used to determine the fatty acid composition of flaxseed oil. This technique enabled us to obtain fatty acid profiles rapidly and with small amounts of seeds. Therefore, a large number of genotype can be tested without sacrificing the seeds. The predominance of the unsaturated fatty acids oleic, linoleic, and linolenic, as we have demonstrated herein is in agreement with the reports of Kenaschuk (1975) and Dybing and Lay (1981).

The main objective of the present study was to evaluate the correlation between DS(X) on 15 flax cultivars and fatty acid composition (Y) of seeds, obtained at harvest from these cultivars. Evaluation of such a correlation is important for several applied and theoretical considerations. First, the quality of flaxseed oil for industrial uses is determined by the percentages of the unsaturated acids oleic, linoleic, and linolenic particularly linolenic acid (Kenaschuk, 1975). Second, most of the known biological activities of flaxseed have been also assigned to linolenic acid (Oomah, 2001). Third, plant fatty acids (FAs) and FAs metabolic pathways have been shown to be of crucial importance in the biology of healthy and infected plants. FAs only serve as a major source of reserve energy but also are important elements of cellular membrane lipids. FAs and their derivatives are included in signalling and modifying normal and disease-related physiologies in infected plants. FAs regulate a range of reactions to biotic and abiotic pressure. For example, polyunsaturated FAs levels in chloroplast membrane affect membrane fluidity and assess the plants capability to acclimatize to temperature stress (Routaboul et al., 2000). Linolenic acid is associated with protein changes in heat-stressed plants (Yamauchi et al., 2008). FAs also control the salt level, drought, and heavy metal tolerance along with wound-induced responses (Tumlinson and Engelberth, 2008) FAs metabolic pathways have critical functions in plant protection against fungal pathogens by providing biosynthetic precursors for cuticular ingredients. FAs and their breakdown products also play more direct roles in inducing various modes of plant defence (Kachroo and Kachroo, 2009).

CONCLUSION

The lack of significant correlation between DS and fatty acid composition in the present study could be attributed to the fact that amounts of fatty acids are highly heritable characters, which indicates that they are mainly governed by genetic factors and therefore, slightly affected by biotic stress resulted from infection by PM. On the other hand, we do not have an immediate biological explanation for the positive and significant correlation between DS and the percentage of the fatty acid Y14. In spite of the significance of this correlation, it seems reasonable to conclude that it was not practically important due to the very low percentage of this fatty acid in the oil.

CONFLICT OF INTEREST

The authors declared that the present study was performed in the absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Add contribution of each author (with abbreviated name) here. For example, WEP designed and performed the experiments and also wrote the manuscript. EW, OA, and IDJ performed animal treatments, flow cytometry experiments, tissue collection, and data analysis. AS and MR designed experiments and reviewed the manuscript. All authors read and approved the final version.

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