

## **Fatty acids compositions of polar and non-polar lipid fractions of wheat leaves inoculated with three brown rust races during progressive pathogenesis**

R. S. SAINI\*, H.K.L. CHAWLA and D. S. WAGLE\*\*

*Department of Chemistry and Biochemistry Haryana Agricultural University Hisar - 125 004, India*

### **Abstract**

The fatty acids composition of the polar and non-polar lipid fractions of wheat leaves was affected due to progressive brown rust infection during early stages of pathogenesis, *i.e.* 0, 12, 24, 36, 48 and 72 h after inoculation. The three races of *Puccinia recondita* differentially affected the composition of saturated and unsaturated fatty acids and their relative occurrence in wheat leaves. The infection of wheat by race 77 resulted in a relative decrease in fatty acid chain length as measured through C<sub>16</sub>:C<sub>18</sub> fatty acid ratio. An increase in the relative degree of unsaturation (18:2/18:3 acids ratio) was recorded in both lipid fractions. Such changes may be taken as one of the earliest characteristics of disease development.

### **Introduction**

Lipids being an important component of the subcellular and cellular membranes and thus play an important role in membrane permeability. Any changes in membrane lipid components, *e.g.* during the process of pathogenesis, are evident through observed changes in membrane permeability (*e.g.* Thatcher 1942, 1943; Hoppe and Heitefuss 1974 a, b, c, 1975). Every membrane has a characteristic lipid composition and a lipid:protein ratio, and alterations of these parameters induce biochemical changes in the membrane most of which are detrimental to its function (*e.g.* Bishop 1983). The present investigation was undertaken to study the effects of brown rust infection on the fatty acid composition of lipid fractions of wheat leaves upon

---

*Received 2 September 1991, accepted 3 January 1994.*

*Acknowledgements:* We are grateful to Dr. R.K. Grover, Shere-Kashmir University of Agriculture and Technology, Srinagar (India) for his kind help in executing the work plan of these studies and to Regional Research Station of IARI, at Flowerdale, Shimla (India) for providing pure cultures of the pathogen. We are also thankful to ICAR and CSIR, New Delhi for providing financial assistance to RSS in the form of Senior Research Fellowships.

\*Present address: Department of Biochemistry, Rajasthan Agricultural University, Agricultural Research Station, Durgapura, Jaipur 302 018, India

\*\*Retired Professor of Biochemistry.

inoculation with three races of the brown rust pathogen, *Puccinia recondita*, during early course of pathogenesis.

### Materials and methods

The wheat, *Triticum aestivum* L. cv. WL711, was grown in the Screen House of the College of Basic Sciences and Humanities as reported earlier (Saini *et al.* 1985). The pathogen races were multiplied and maintained on a universally susceptible wheat cultivar, Agra Local. Second and third leaves of 25-d-old plants were inoculated by uniformly pasting the spore mass powder of the pathogen on the lower surface of the leaves individually (Saini *et al.* 1988). The samplings were made by harvesting the leaves before inoculation (0 h sampling) and at 12, 24, 36, 48 and 72 h after inoculation.

Lipids were extracted and samples preserved according to Hoppe and Heitefuss (1974b). The combined methanol-chloroform layers were evaporated to dryness at 40 °C. Lipid residue was dissolved in chloroform and dried under vacuum after filtration through a sintered glass funnel (pore size G-3).

Fractionation of lipids into polar and non-polar fractions was accomplished according to the method of Nichols (1964). The polar lipids were obtained by evaporating the methanol phase and the petroleum ether phase contained the non-polar lipids.

For determination of fatty acid composition of lipid fractions the methyl esters were prepared by the method of Luddy *et al.* (1960) taking 20 mg of each liquid lipid sample for preparing the esters which were obtained in lower layer of carbondisulfide. Gas liquid chromatography separation of the methyl esters was performed at *Hewlett-Packard Gas Chromatograph Model No. 65730A* equipped with a flame ionization detector (FID). The operation temperatures were: oven - 190, detector - 300 and injector - 250 °C. The various fatty acids during their GLC separation had a retention time of 2.56, 3.50, 5.12, 6.10, 8.07 and 11.03 min for palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), linoleic (18:2) and linolenic (18:3) acids, respectively.

### Results and discussion

Linolenic (18:3) an unsaturated fatty acid (USFA) was found to be the major fatty acid present in the polar and non-polar lipid fractions of the wheat leaves while another unsaturated fatty acid with two double bonds *i.e.* linoleic (18:2) and a saturated fatty acid *i.e.* palmitic (16:0) ranked the next two main fatty acids present in the lipid fractions (Table 1a and 1b).

The saturated fatty acids (SFA) content of polar lipid fractions in wheat leaves infected with race 63 was gradually decreasing at various stages with some intermittant increases after 24 h and 48 h, while the infection by race 104 firstly showed a slight decrease in SFA content of polar lipid fraction after 12 h, increase

CHANGES IN WHEAT LEAF LIPIDS DURING PATHOGENESIS

Table 1a. Relative composition of various fatty acids of polar lipid fraction of wheat leaves inoculated with *Puccinia recondita*, races 63, 104 and 77.

Time after inoculation [h]	Race	Fatty acids [%]						
		Lower	Palmitic (16:0)	Palmitoleic (16:1)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)	Linolenic (18:3)
0		0.02	8.75	1.75	0.40	1.75	7.00	80.26
12	63	0.20	6.68	0.03	1.63	0.03	6.07	85.33
	104	0.09	8.09	1.62	0.37	1.32	7.58	80.93
	77	0.06	6.40	2.26	0.60	0.90	6.64	83.05
24	63	0.07	8.82	1.76	0.58	1.02	7.64	80.07
	104	0.10	8.18	0.65	0.98	2.45	5.73	81.88
	77	0.06	7.36	0.06	0.13	0.78	7.36	84.21
36	63	0.18	7.15	1.84	0.47	0.87	7.86	81.59
	104	0.06	9.39	2.10	0.48	0.90	7.58	79.47
	77	0.06	9.00	0.12	0.12	1.12	7.00	82.55
48	63	0.02	9.79	0.02	0.04	1.22	7.13	81.59
	104	0.16	9.86	0.49	2.36	2.30	9.20	75.60
	77	0.10	8.50	1.27	0.04	1.27	3.82	84.57
72	63	0.30	7.68	0.07	0.14	1.81	5.43	84.55
	104	0.02	8.75	1.75	0.43	1.75	7.00	80.26
	77	0.88	8.54	5.33	0.14	0.71	4.57	79.82

Table 1b. Compositional relationships of fatty acids of polar lipid fraction of wheat leaves inoculated with *Puccinia recondita*.

Time after inoculation [h]	Race	Compositional relationships					
		SFA: USFA	SFA: linolenic	USFA: linolenic	total: linolenic	linoleic: linolenic	C <sub>16</sub> :C <sub>18</sub>
0		1:9.91	1:8.77	1:0.88	1:0.80	1:11.46	1:8.51
12	63	1:11.00	1:20.26	1:0.93	1:0.85	1:14.05	1:13.86
	104	1:10.80	1:9.56	1:0.88	1:0.80	1:10.67	1:9.28
	77	1:13.26	1:11.86	1:0.89	1:0.83	1:12.50	1:10.53
24	63	1:9.62	1:8.51	1:0.88	1:0.80	1:10.48	1:8.36
	104	1:9.79	1:8.93	1:0.91	1:0.81	1:14.28	1:10.31
	77	1:12.33	1:12.42	1:0.91	1:0.84	1:11.44	1:12.46
36	63	1:12.09	1:10.70	1:0.88	1:0.81	1:10.36	1:9.98
	104	1:9.12	1:8.05	1:0.88	1:0.79	1:10.48	1:7.69
	77	1:9.95	1:8.97	1:0.90	1:0.82	1:11.79	1:9.95
48	63	1:9.15	1:8.30	1:0.90	1:0.81	1:11.44	1:9.17
	104	1:7.16	1:6.18	1:0.86	1:0.75	1:8.21	1:8.64
	77	1:10.69	1:9.23	1:0.93	1:0.84	1:22.24	1:9.22
72	63	1:11.74	1:10.81	1:0.92	1:0.84	1:15.57	1:11.86
	104	1:9.88	1:8.74	1:0.88	1:0.80	1:11.46	1:8.51
	77	1:10.42	1:9.20	1:0.88	1:0.79	1:17.48	1:6.15

after 24 and 48 h, but again decline after 72 h of infection (Table 2). Race 77 caused a general decrease in SFA content of polar lipids in exception at 36 h where it was maximum. Slight changes in USFA concentrations of polar lipid fractions were noted at various stages of infection caused by all the three races and it ranged from 89.96 % at 48 h to 92.16 % at 36 h (race 63), from 87.59 % at 48 h to 91.45 % at 12 h (race 104) and from 90.53 % at 72 h to 92.85 % at 24 h stage (race 77).

Infection by race 63 caused little changes in SFA concentration in non polar fraction (Table 2) at various stages except at 36 h and 72 h stages. Infection by race 104 caused sharp decrease in SFA concentration at 12 h and 36 h while at the remaining stages the values were a bit lower than that at 0 h stage. Infection by race 77 caused increase in SFA concentration at 12 h and 24 h but later the decrease. The USFA concentration gradually decreased at successive stages with increases at 24 h and 48 h stages but still was with a lower profile. While race 104 caused a sharp increase at 12 h but later on decrease toward 48 h and once again increase toward 72 h stage and race 77 caused no change in USFA concentration at 12 h but the value declined at the next two stages thereafter sharp increments were noticed in the later two stages of determination (Table 2).

Results obtained with artificial liposomal membranes and natural systems indicate that the permeability of the membrane toward water-soluble non electrolytes depends upon unsaturation and the chain length of the membranal fatty acids (De Gier *et al.* 1968, Mc Elhaney *et al.* 1970 and Romin *et al.* 1972). The permeability of the natural membrane system is found to be markedly increased with the degree of unsaturation and decreasing chain length of the fatty acids present in the lipids (Hoppe and Heitefuss 1974c, Hoppe and Heitefuss 1975).

Also in the present investigation in case of infection by race 77 (*i.e.* susceptible-interaction) and at 72 h stage in particular a decrease in fatty acid chain length was evident as measured through C<sub>16</sub>:C<sub>18</sub> fatty acid ratios *i.e.* the ratios were, 1:4.00 at 0 h, 1:2.77 at 12 h, 1:3.14 at 24 h, 1:2.97 at 36 h, 1:4.83 at 48 h and 1:2.75 at 72 h in non polar lipid fraction and in polar lipid fraction it was found to be 1:8.51, 1:10.53, 1:12.46, 1:9.95, 1:9.22 and 1:6.15 at successive stages (Tables 3a and 3b). Similarly the degree of unsaturation *i.e.* linoleic:linolenic acid ratios also seemed to be increasing toward 72 h stage which may again indicate the increased permeability of the tissue membranes with the advancement of the rust infection. We have already reported about the increased degradation of the membrane lipids and subsequent changes in permeability leakage of the various solutes in the comparatively compatible-interactions as evidenced by increased activities of some of the enzymes of lipid metabolism (Saini *et al.* 1989, 1990).

In general the assessment of the results on the fatty acid composition of the lipid moieties present in the polar and non-polar lipid fractions may be characterised in relation to resistance or susceptibility *i.e.* decreasing chain length and the degree of unsaturation of the fatty acids indicated the susceptible nature of the interaction by race 77 for favour of disease development and establishment in an easier way.

Table 2. Relative composition of saturated (SFA) and unsaturated (USFA) fatty acids of polar and non polar lipid fractions of wheat leaves infected with three brown rust races.

Time after inoculation [h]	race 63						race 104						race 77					
	polar SFA		non polar SFA		USFA		polar SFA		non polar SFA		USFA		polar SFA		non polar SFA		USFA	
0	9.15	90.76	21.83	75.84	9.15	90.76	21.83	75.84	9.15	90.76	21.83	75.84	9.15	90.76	21.83	75.84	9.15	90.76
12	8.31	91.41	21.72	73.87	8.46	91.95	15.27	82.12	7.00	92.85	22.83	75.84	7.00	92.85	22.83	75.84	7.00	92.85
24	9.40	90.49	21.11	75.29	9.16	89.71	19.81	76.56	7.49	92.41	21.75	73.83	7.49	92.41	21.75	73.83	7.49	92.41
36	7.62	92.16	23.34	73.58	9.87	90.05	14.52	73.58	9.82	90.79	21.68	73.32	9.82	90.79	21.68	73.32	9.82	90.79
48	9.83	89.96	22.02	74.55	12.22	87.59	20.46	73.61	8.54	91.33	16.76	79.43	8.54	91.33	16.76	79.43	8.54	91.33
72	7.82	91.86	23.89	72.19	9.18	90.76	20.42	77.22	8.68	90.53	16.75	79.94	8.68	90.53	16.75	79.94	8.68	90.53

Table 3a. Relative percent composition of various fatty acids of non-polar lipid fraction of wheat leaves inoculated with *Puccinia recondita*.

Time after inoculation [h]	Race	Fatty acids						
		Lower	Palmitic (16:0)	Palmitoleic (16:1)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)	Linolenic (18:3)
0		2.29	18.39	1.14	3.44	6.89	17.24	50.57
12	63	4.34	20.86	0.86	0.86	8.69	20.86	43.46
	104	2.54	15.27	1.90	trace	10.18	25.47	44.47
	77	2.84	21.41	4.27	1.42	8.54	14.99	46.42
24	63	3.44	20.68	1.29	0.43	5.16	25.85	43.09
	104	3.60	18.01	1.80	1.80	10.81	14.41	49.54
	77	3.32	22.21	1.09	0.54	13.23	17.77	41.65
36	63	2.92	20.84	0.40	2.50	15.06	18.39	39.73
	104	2.21	12.63	3.15	1.89	5.52	17.69	56.87
	77	4.96	18.38	5.51	3.30	13.23	14.88	39.70
48	63	3.27	19.65	1.63	2.37	5.12	13.71	54.09
	104	5.84	15.47	0.46	2.91	4.38	11.70	57.07
	77	4.11	16.44	trace	0.32	5.13	12.33	61.65
72	63	3.55	23.65	trace	0.22	7.10	18.93	46.16
	104	2.26	18.16	1.12	2.26	6.81	13.62	55.67
	77	3.21	15.47	10.31	1.28	3.86	15.47	50.30

Table 3b. Compositional relationships of fatty acids of non-polar lipid fraction of wheat leaves inoculated with *Puccinia recondita*.

Time after inoculation [h]	Race	Compositional relationships					
		SFA: USFA	SFA: linolenic	USFA: linolenic	total: linolenic	linoleic: linolenic	C <sub>16</sub> :C <sub>18</sub>
0		1:3.47	1:2.31	1:0.66	1:0.50	1:2.93	1:4.00
12	63	1:3.40	1:2.00	1:0.58	1:0.43	1:2.08	1:3.40
	104	1:5.37	1:2.91	1:0.54	1:0.44	1:1.74	1:4.67
	77	1:3.32	1:2.03	1:0.61	1:0.46	1:3.09	1:2.77
24	63	1:3.56	1:2.04	1:0.59	1:0.43	1:1.66	1:3.39
	104	1:3.86	1:2.50	1:0.64	1:0.49	1:3.43	1:3.86
	77	1:3.24	1:1.83	1:0.56	1:0.41	1:2.34	1:3.14
36	63	1:3.15	1:1.70	1:0.53	1:0.39	1:2.16	1:3.56
	104	1:5.73	1:3.91	1:0.68	1:0.56	1:3.21	1:5.19
	77	1:3.38	1:1.83	1:0.54	1:0.39	1:2.66	1:2.97
48	63	1:3.38	1:2.45	1:0.72	1:0.54	1:3.94	1:3.53
	104	1:3.59	1:3.93	1:0.77	1:0.57	1:4.87	1:4.22
	77	1:4.73	1:3.67	1:0.77	1:0.61	1:5.00	1:4.83
72	63	1:3.02	1:1.93	1:0.63	1:0.46	1:2.43	1:3.05
	104	1:3.78	1:2.72	1:0.72	1:0.55	1:4.08	1:4.06
	77	1:4.77	1:3.00	1:0.62	1:0.50	1:3.25	1:2.75

## References

- Bishop, D.G.: Functional role of plant membrane lipids. - In: Thomson, W.W., Mudd, J.B., Gibbs, M. (ed.): *Biosynthesis and Function of Plant Membrane Lipids*. Pp. 81-103. University of California, Riverside 1983.
- DeGier, J., Mandersloof, J.G., Van Deenen, L.L.M.: Lipid composition and permeability of liposomes. - *Biochim. biophys. Acta* **150**: 666-675, 1968.
- Hoppe, H.H., Heitefuss, R.: Permeability and membrane lipid metabolism of *Phaseolus vulgaris* infected with *Uromyces phaseoli*. I. Changes in the efflux of cell constituents. - *Physiol. Plant Pathol.* **4**: 5-9, 1974a.
- Hoppe, H.H., Heitefuss, R.: Permeability and membrane lipid metabolism of *Phaseolus vulgaris* infected with *Uromyces phaseoli*. II. Changes in lipid concentration and <sup>32</sup>P incorporation into phospholipids. - *Physiol. Plant Pathol.* **4**: 11-23, 1974b.
- Hoppe, H.H., Heitefuss, R.: Permeability and membrane lipid metabolism of *Phaseolus vulgaris* infected with *Uromyces phaseoli*. III. Changes in relative concentration of lipid bound fatty acids and phospholipase activity. - *Physiol. Plant Pathol.* **4**: 25-35, 1974c.
- Hoppe, H.H., Heitefuss, R.: Permeability and membrane lipid metabolism of *Phaseolus vulgaris* infected with *Uromyces phaseoli*. IV. Phospholipids and phospholipid fatty acids in healthy and rust infected bean leaves resistant and susceptible to *Uromyces phaseoli*. - *Physiol. Plant Pathol.* **5**: 263-271, 1975.
- Luddy, F.E., Barford, R.A., Reimensch-Rieder, R.W.: Direct conversion of lipid components to their fatty acid methyl esters. - *J. amer. Oil chem. Soc.* **37**: 447-451, 1960.
- McElhaney, R.N., De Gier, J., Van Deenen, L.L.M.: The effect of alterations in fatty acid composition and cholesterol content on the permeability of *Mycoplasma laidlawii* B cells and derived liposomes. - *Biochim. biophys. Acta* **219**: 245-247, 1970.
- Nichols, B.W.: Separation of plant phospholipids and glycolipids. - James, A.I., Morris, L.J. (ed.): *New Biochemical Separations*. Pp. 321-337. Van Nostrand Co., London 1964.
- Romin, J.C., Van Golde, L.M.G., Mc Elhaney, R.N., Van Deenen, L.L.M.: Some studies on the fatty acid composition of total lipids and phosphatidyl glycerol from *Acholepsma laidlawii* B and their relation to the permeability of the intact cells of the organism. - *Biochim. biophys. Acta* **280**: 22-32, 1972.
- Saini, R.S., Arora, Y.K., Wagle, D.S.: Soluble proteins and multiple forms of peroxidase in growing points of wheat plants in relation to loosesmut. - *Biochem. Physiol. Pflanz.* **180**: 239-245, 1985.
- Saini, R.S., Chawla, H.K.L., Wagle, D.S.: Leakage of electrolytes and phenols and altered activity profile of peroxidase and its isoenzymes from wheat leaves inoculated with *Puccinia recondita*. - *Biol. Plant.* **30**: 387-392, 1988.
- Saini, R.S., Chawla, H.K.L., Wagle, D.S.: Lipoxygenase activity, total lipid content and leaf mass leaching pattern of wheat leaves inoculated with brown rust, *Puccinia recondita*. - *Biol. Plant.* **31**: 207-212, 1989.
- Saini, R.S., Chawla, H.K.L., Wagle, D.S.: Catabolic activity of two phosphoric diester hydrolases in wheat leaves inoculated with brown rust, *Puccinia recondita*. - *Biol. Plant.* **32**: 313-318, 1990.
- Thatcher, F.S.: Further studies of osmotic and permeability relations in parasitism. - *Can. J. Res. Sect. C* **20**: 283-311, 1942.
- Thatcher, F.S.: Cellular changes in relation to rust resistance. - *Can. J. Res. (Sect. C)* **21**: 151-172, 1943.
- Wheeler, H., Hanchey, P.: Permeability phenomenon in plant disease. - *Annu. Rev. Phytopathol.* **6**: 337-350, 1968.