*J. Phytol. Res.* 7 (2): 147-151, 1994

# FATTY ACID COMPOSITION OF SPORE AND MYCELIAL LIPIDS OF *NEOVOSSIA INDICA*, KARNAL BUNT OF WHEAT UNDER DIFFERENT SETS OF CULTURAL CONDITIONS

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Linoleic acid followed by palmitic acid were the major fatty acids in both the spore and mycelial lipids of *Neovossia indica*. The fatty acids with chain length less than $C_{10}$ were present only in very minor quantities. Different lipid fractions obtained from cultures grown at 10°C had a higher content of linolenic acid as compared to those grown at 20°C Arachidic acid, a long chain fatty acid seemed to be very actively synthesised during the early stages of mycelial growth, i.e., 5 to 8 days and its rate of synthesis declined subsequently.

**Keywords:** Fatty acid; Karnal bunt; *Neovossia indica*; wheat.

## Introduction

Among the smut diseases of wheat, Karnal bunt (*Neovossia indica* ) is the most serious one as till now there are no true resistant varieties against this organism. The spores of the rust and smut fungi, which cause the diseases of wheat, have been reported to contain abundant quantities of lipids, generally upto 10% or more in mycelium on dry weight basis. These lipids are also considered as reserve material which may be converted to energy and carbon skeletons during growth and reproduction. The function of the lipids in the vegetative hyphae of fungi is not well understood. The present paper reports on fatty acid composition of spore and mycelial lipids fluctuations occurring under different cultural conditions.

## Materials and Methods

The culture of *Neovossia indica* was maintained by quarterly transfer on the potato dextrose agar medium. This medium without agar was also used for raising the inocula throughout the course of this study.

*Neovossia indica* was grown in 250 ml Erlenmeyer flasks containing 50 ml of liquid medium. The organism was grown for 5,8,11,14 and 17 days as still culture. The effect of temperature was studied at 10 and 20°C.

Total lipids from spores and mycelium were extracted by the method of Folch *et al* [1]. Technique of thin layer chromatography was employed for the purification of different lipid classes and identification and quantitaion of individual lipid components as already reported[2-4].

The fatty acids composition was determined using NUCON 5700 GAS Chromatograph, after esterification of lipids by the method of Luddy *et al.*[5] The conditions for the separation were: oven temperature 180°C, injector temperature 205°C, F.I.D.temperature 250°C, carrier gas nitrogen with flow rate 30 ml/min, hydrogen

flow rate 30 ml/min, air flow rate 300 ml/min, flame attenuation 64 and recorder chart speed 10 mm/min. The peaks were identified by their retention time and also by using standard fatty acyl esters as internal standard.

**Results and Discussion**

*Fatty acid composition of spore lipid fractions:* Linolenic acid was the major fatty acid present in various lipid fractions; monoglycerides, diglycerides, sterols (MDS), free fatty acids (FFA), sterol esters and hydrocarbons(SEH) triacylglycerols (TG) and total phospholipids and constituted 78.97, 41.24, 47.47, 76.74 and 61.30% respectively in these fractions (Table 1). The corresponding values for palmitic acid were 13.91, 21.30, 19.19, 16.91 and 24.28%, respectively, in these fractions.

Fatty acids with chain length C10 and below were present in traces in some fractions and constiuted less than 4% in others. Arachidic acid constituted 13.74% in FFA and 11.53% in PL fractions. Unsaturated fatty acids were preferentially concentrated in the neutral lipid fractions than in the polar lipid fractions.

Tullock and Ledingham[6,7] reported the fatty acid composition of spores produced by over 40 Basidiomycetes fungi and observed considerable variations among the lipid composition of different species. It was found that linoleic acid was the most abundant acid in teliospores and basidiospores which was present in relative abundance of over 62%. The predominant saturated fatty acid found in these spores was palmitic acid.

*Effect of incubation period on fatty acid composition of mycelial lipid fractions* : The fatty acid composition of the different lipid fractions in lipids of mycelium grown for 5,8,11,14 and 17 days are presented in Table 2. Palmitic acid was the major fatty acid among saturated fatty acids at all stages of the growth and in all the lipid fractions isolated. The most predominant fatty acid among the unsaturated fatty acids was linolenic acid. In the lipid fractions of 5 days old culture, the C12:O and C14:0 acids collectively constituted less than 10% of the total fatty acids. Lower fatty acids with chain length of C10 and below were present only in minor quantities. The synthesis of arachidic acid seemed to be very active during the early stages of growth.

Lennarz *et al.*[8] reported that the fatty acids of bacterial lipids in general exhibit an increased saturation with the advancement of culture age. They observed high proportions of oleic acid and low proportions of methyl stearic acid in *Mycobacterium phlei* as the culture passed from active growth to stationary phase. Verma and Khuller[9] studied the acyl chains of phospholipids and their variations with age in Str*eptomyces griseus* and reported that a decrease takes place in saturated fatty acid levels with a simultaneous increase in unsaturated fatty acids.

*Effect of growth temperature on fatty acid composition of mycelial lipid fractions* : The temperature is one of the most important factors affecting the growth and metabolism of microorganisms. Early studies of Johnson[10] regarding the effect of temperature on the lipids of microorganisms showed that at low temperature the lipids contained higher proportion of unsaturated

acids than at optimum temperature.

Comparison of the fatty acid composition of the mycelial lipid fractions obtained from mycelial cultures grown at two different temperatures, 10°C and 20°C revealed a considerable increase in the synthesis of linolenic acid in almost all the lipid fractions at lower temperature (Table 2 and 3). At the temperature 10°C the synthesis of oleic acid was also found to be slightly enhanced. The values for the total unsaturated fatty acids for most of the lipid fractions, were found to be higher at 10°C as compared to 20°C. The increased unsaturation at lower temperature might be due to an increase in the acitivity of enzymes fatty acid desaturase. It is a well established fact that oxygen is required during the dehydrogenation of fatty acids and also decrease in temperature increases the oxygen solubility which in turn enhances the rate of unsaturation. The maximum increase in unsaturation was noticed in PG+CL fraction where the value of 41.16% for total unsaturated fatty acids at 20°C was increased to 62.80% at 10°C.

Marr and Ingraham[11] while studying the effect of temperature on the fatty acid composition of E. *coli* grown over the range of 43°C to 10°C reported that proportions of hexadecanoic and octadecanoic acids increased continuously while palmitic acid decreased with the decreasing temperature of growth medium, resulting in a fairly high degree of unsaturation of total fatty acids. Similar observations have also been reported by Gaughren[12], working on Bac*illus subtilis*, Long and Williams[13] working on *B. stearothermophiles* and Kates and Baxter[14] while working on three psychrophilic yeasts of genus *Candida* grown at lower and higher temperatures than the optimum growth temperatures.

By comparing the fatty acid composition of fungal spores with that of the mycelial lipids it can be concluded that though qualitatively, it is similar, however, the incubation temperature influenced the fatty acid composition of mycelial lipids. The main effect noticed was that the synthesis of more unsaturated fatty acids had taken place in the fungus grown at lower temperature.

**Table 1.** Fatty acid composition of spore liquid fractions of *Neovossia indica.*

| Lipid | C10 and | Relative percentage of fatty acids | | | | | | |
|---|---|---|---|---|---|---|---|---|
| frac-tions | below | C12:0 | C14:0 | C16:0 | C18:1 | C18:2 | C18:3 | C20:0 |
| MDS | 0.44 | 0.41 | - | 13.91 | 1.36 | 78.97 | 4.91 | - |
| FFA | 2.41 | 5.50 | 6.41 | 21.30 | 5.27 | 41.24 | 4.12 | 13.74 |
| TG | 0.90 | 1.61 | 0.31 | 16.91 | - | 76.74 | - | 3.52 |
| SEH | 4.44 | 11.76 | 3.61 | 19.19 | 4.34 | 47.47 | 5.16 | 4.02 |
| PL | 1.87 | - | - | 24.28 | - | 61.30 | 1.01 | 11.53 |

MDS = Monoglycerides Diglycerides and free sterols; FFA = Free fatty acids; TG = Triglycerides; SEH = Sterol esters and Hydrocarbons; PL = Polar lipids.

**Table 2.** Fatty acid composition of mycelial lipid fractions of *Neovossia indica* after 5, 8, 11, 14 and 17 days of incubation grown at 20°C as still cultures.

| Lipid fractions | $C_{10}$ and below | $C_{12:0}$ | $C_{14:0}$ | Relative percentage of fatty acids $C_{16:0}$ | $C_{18:1}$ | $C_{18:2}$ | $C_{18:3}$ | $C_{20:0}$ |
|---|---|---|---|---|---|---|---|---|
| **5 DAYS** | | | | | | | | |
| MDS* | 2.14 | 2.30 | 2.76 | 46.86 | - | 45.94 | - | - |
| FFA* | 6.35 | 6.49 | 4.62 | 30.30 | 5.77 | 45.02 | 1.44 | - |
| TG* | 0.24 | 1.85 | 0.61 | 17.55 | 1.12 | 78.08 | - | 0.54 |
| SEH* | 6.39 | 0.82 | 3.67 | 24.49 | 6.09 | 53.74 | 2.27 | 2.49 |
| PA | 1.05 | 2.36 | 3.60 | 42.07 | - | 50.92 | - | - |
| PI | 5.33 | 4.00 | 4.00 | 18.00 | - | 32.00 | - | 36.67 |
| PC | 1.42 | 2.40 | 3.20 | 46.73 | - | 45.20 | - | 1.04 |
| PE | 1.41 | 2.43 | 1.30 | 35.10 | 1.95 | 57.14 | - | 0.65 |
| PG+CL | 2.42 | 2.47 | 3.29 | 28.82 | - | 62.99 | - | - |
| SG+P | 6.44 | 3.27 | 3.47 | 27.04 | 5.45 | 47.41 | - | 6.93 |
| **8 DAYS** | | | | | | | | |
| MDS | 1.47 | 4.74 | 0.55 | 25.19 | 1.93 | 59.95 | 0.41 | 5.76 |
| FFA | 7.05 | 8.76 | 12.57 | 18.09 | 5.71 | 38.29 | - | 9.52 |
| TG | 7.27 | 8.06 | 2.01 | 24.37 | - | 58.01 | - | 0.27 |
| SEH | 7.31 | 4.95 | 4.33 | 14.80 | 4.95 | 54.10 | 3.71 | 5.56 |
| PA | 1.55 | 3.43 | 0.46 | 42.25 | - | 51.55 | - | 0.76 |
| PI | 0.60 | 1.45 | 1.56 | 10.09 | 1.25 | 83.84 | 0.80 | 0.40 |
| PC | 1.60 | 3.71 | 6.16 | 25.18 | 11.03 | 37.93 | 10.71 | 4.22 |
| PE | 0.33 | 0.77 | 0.28 | 23.15 | - | 75.46 | - | - |
| PG+CL | 9.69 | 10.05 | 13.97 | 25.14 | - | 41.16 | - | - |
| SG+P | 2.89 | 5.49 | 1.26 | 30.95 | 3.18 | 55.39 | 0.82 | - |
| **11 DAYS** | | | | | | | | |
| MDS | 0.76 | 1.42 | 1.07 | 20.04 | 1.20 | 73.37 | - | 2.14 |
| FFA | 1.88 | 1.74 | 2.09 | 39.78 | 2.51 | 40.19 | 1.05 | 10.75 |
| TG | 0.30 | 0.95 | 0.02 | 32.95 | - | 65.45 | - | 0.33 |
| SEH | 4.37 | 8.66 | 5.42 | 41.91 | - | 35.02 | 1.15 | 3.46 |
| PA | 3.24 | 1.39 | 2.16 | 37.04 | - | 54.01 | 1.39 | 0.77 |
| PI | 0.26 | 0.64 | 0.72 | 47.14 | - | 51.04 | - | 0.19 |
| PC | 0.70 | 0.81 | 0.83 | 11.37 | 1.51 | 81.35 | 0.65 | 2.78 |
| PE | 3.68 | 1.28 | 4.27 | 43.16 | - | 45.81 | 1.79 | - |
| PG+CL | 0.84 | 1.40 | 0.75 | 32.33 | - | 64.69 | - | - |
| SG+P | 1.34 | 1.18 | 1.18 | 17.14 | - | 71.08 | 2.96 | 5.12 |
| **14 DAYS** | | | | | | | | |
| MDS | 2.24 | 3.08 | 1.17 | 38.22 | - | 53.90 | - | 1.40 |
| FFA | 2.80 | 2.83 | 2.02 | 26.58 | 5.14 | 48.72 | 0.47 | 11.44 |
| TG | 0.50 | 1.85 | 0.42 | 28.63 | - | 67.78 | - | 0.82 |
| SEH | 7.30 | 14.55 | 11.38 | 20.76 | 3.54 | 33.29 | 3.57 | 5.59 |
| PA | 1.35 | 2.93 | 3.49 | 36.48 | 2.18 | 51.72 | 0.81 | 1.04 |
| PI | 2.03 | 0.39 | 4.90 | 19.13 | 1.31 | 71.25 | 0.98 | - |
| PC | 3.56 | 3.27 | 3.31 | 38.68 | - | 48.59 | 2.03 | 0.56 |
| PE | 2.38 | 4.10 | 2.97 | 35.69 | 2.59 | 49.73 | 2.53 | - |
| PG+CL | 6.25 | 1.67 | 3.75 | 29.17 | - | 55.00 | - | 4.17 |
| SG+P | 3.72 | 6.47 | 4.37 | 24.27 | 2.59 | 48.06 | - | 10.52 |
| **17 DAYS** | | | | | | | | |
| MDS | 0.92 | 1.32 | 0.47 | 31.37 | - | 63.27 | - | 2.64 |
| FFA | 3.30 | 1.49 | 2.25 | 30.91 | 3.70 | 45.82 | 3.01 | 9.52 |
| TG | 0.87 | 1.98 | 0.60 | 37.59 | - | 57.60 | - | 1.35 |
| SEH | 2.32 | 8.20 | 2.15 | 34.15 | - | 51.95 | - | 1.23 |
| PA | 1.93 | 3.55 | 1.03 | 60.04 | - | 31.63 | - | 1.81 |
| PI | 2.71 | 5.35 | 12.76 | 24.31 | 3.80 | 44.25 | 5.47 | 1.34 |
| PC | 8.45 | 2.67 | 3.92 | 37.95 | - | 41.00 | 4.80 | 1.20 |
| PE | 3.40 | 3.32 | 6.08 | 43.10 | 2.39 | 40.12 | 1.59 | - |
| PG+CL | 2.42 | 4.06 | 2.18 | 26.13 | 6.86 | 54.60 | 3.74 | - |
| SG+P | 1.61 | 3.28 | 1.00 | 40.50 | - | 44.82 | 4.01 | 4.78 |

*As in Table 1

PA = Phosphatidic acid ; PI = Phosphatidylinositol ; PC = phosphatidyl choline; PE = Phosphatidyl ethanolamine; PG+CL = Poltglycerophosphatides and cardiolipins.

**Table 3.** Fatty acid composition of mycelial lipid fractions of *Neovossia indica* after 8 days of incubation grown at 10°C as still cultures.

| Lipid fractions | Relative percentage of fatty acids | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | $C_{10}$ and below | $C_{12:0}$ | $C_{14:0}$ | $C_{16:0}$ | $C_{18:1}$ | $C_{18:2}$ | $C_{18:3}$ | $C_{20:0}$ |
| MDS | 3.96 | 2.64 | 2.64 | 40.73 | - | 40.45 | 2.31 | 7.26 |
| FFA | 4.15 | 1.90 | 2.18 | 29.10 | 5.34 | 43.52 | 6.66 | 7.14 |
| TG | 0.64 | 2.21 | 1.16 | 35.18 | 2.49 | 56.77 | | 1.55 |
| SEH | 1.52 | 1.91 | 3.82 | 38.93 | - | 47.76 | 2.62 | 3.43 |
| PA | 1.50 | 3.90 | 4.95 | 31.05 | 4.50 | 49.23 | 3.07 | 1.80 |
| PI | 2.66 | 3.38 | 4.06 | 36.22 | - | 46.28 | 7.40 | - |
| PC | 4.07 | 1.77 | 4.91 | 36.15 | 4.47 | 44.01 | 3.14 | 1.47 |
| PE | 0.92 | 1.41 | 1.28 | 17.19 | - | 79.20 | - | - |
| PG+CL | 3.54 | 0.96 | 6.02 | 25.06 | 8.35 | 51.40 | 3.05 | 1.61 |
| SG+P | 4.05 | 2.80 | 3.49 | 28.14 | 5.31 | 40.89 | 7.23 | 8.07 |

From the results of the present investigation, it is concluded that by changing the cultural conditions of the organism, its fatty acid composition can be manipulated. It is hoped that the results reported here may help in controlling the lipid metabolism in *Neovossia indica,* which may help the plant breeders in understanding the mechanism for the regulation of germination process and production of sporidia in *Neovossia indica* and also in understanding the disease cycles. This, in turn, will help to evolve the control measures used to reduce the severity of infestation by Karnal bunt.

**Acknowledgements**

One of the authors (JSA) is highly thankful to the United State Department of Agriculture for the research grant under Cooperative Agricultural Research Grant Programme for the PL-480 research project, for which the author is Principal Investigator. Authors are also thankful to Dr.K.L.Bajaj, Senior Biochemist-cum-Head, for his valuable suggestions in the preparation of the manuscript.

**References**

1. Folch J, Lees M and Slounestanley 1957, J.*Biol. Chem.* **226** 497
2. Bhatia I S and Arneja J S 1978, J.*Sci.Fd.Agric.* **29** 611
3. Bhatia I S and Arneja J S 1978, J.*Sci.Fd.Agric.* **29** 619
4. Arneja J S 1988, Acta *Mycologica* **23** (1) 149
5. Luddy F E, Barford R A, Herb S F and Magidman 1968, J.m.*Oil Chem. Sec.* **45** 519
6. Tullock A P and Ledingham G A 1960, Can. *J. Microbiol.* **6** 425
7. Tullock A P and Ledingham G A 1962, Can. *J. Microbiol.* **8**, 379
8. Lennarz, W J, Scheurbrandt G and Bloch K 1962, J.*Biol. Chem.* **237** 664
9. Verma J N and Khuller G K 1984, Adv. *in Lipid Res.* **20** 257
10. Johnson F H 1957, In : Microbial *Ecology* (R.E.C.Williams and C.C.Spicer eds.) **2** : 134-167. Cambridge University Press, London and New York.
11. Marr G A and Ingram J L 1962, J.*Bacteriol.* **84** 1260
12. Gaughran E R L 1949, J. *Gen. Physoil.* **32** 313
13. Long S K and Williams O B 1960, J. *Bacteriol.* **79** 629
14. Kates M and Baxter R M 1962, Can.*J.Biochem. Physiol.* **40** 1213.