Phytochemistry, 1977, Vol. 16, pp. 1481–1484. Pergamon Press. Printed in England.

CHARACTERISATION OF MITOCHONDRIAL FRACTIONS ISOLATED FROM FOUR GENOTYPES OF WHEAT COLEOPTILES

AÏDA LABIB* and J. C. KADER†

*Laboratoire d'Amélioration des Plantes, Université de Paris-Sud, 91405 Orsay France;
†Laboratoire de Physiologie Célulaire, ERA 323, Université Pierre et Marie Curie, 12, rue Cuvier, 75005 Paris, France

(*Revised received* 11 *April* 1977)

Key Word Index—*Triticum aestivum*; *Triticum timopheevi*; Gramineae; wheat; heterosis; male sterility; fatty acids; polypeptides; ADP; O ratio.

Abstract—Mitochondrial fractions were isolated at various stages of germination, from wheat coleoptiles of 4 different genotypes: WRH, Capitole, male-sterile Capitole (MSC) and F_1 hybrid obtained by crossing MSC with WRH. The ADP:O values were the highest in F_1 and in MSC and decreased during germination. The oxygen consumption was weaker in F_1 and in WRH than in the other genotypes. Quantitative differences in polypeptides exist between the different genotypes. On germination, increase in the polypeptides of high MW was more rapid in F_1 than in the other genotypes. The fatty acids of the mitochondria of F_1 and MSC are characterized by an abundance of linoleic acid. The content of this fatty acid decreased during germination more rapidly in F_1 and in MSC than in the others. The cytoplasm of *Triticum timopheevi* has a marked influence on mitochondria of MSC and F_1. This cytoplasm confers better oxidative properties, which can be correlated with changes in their biochemical constituents. However the F_1 hybrid differs from the other genotypes in many biochemical features.

INTRODUCTION

Little information is available concerning the relationship between nuclear and/or cytoplasmic information and biochemical properties of mitochondria isolated from higher plants [1, 2]. The studies may be conveniently performed with wheat coleoptiles. In the present work, we used two inbred lines (WRH 69002 and Capitole), a cytoplasmic male sterile form of Capitole and the F_1 hybrid obtained by crossing MSC (♀) with WRH (♂). This F_1 hybrid is fertile, its *T. timopheevi* cytoplasm (sterile) being restored by nuclear genes present in the male parent. This paper reports striking differences between the biochemical properties (oxidative capacities, protein and lipid composition) of mitochondrial preparations isolated from these 4 genotypes of wheat coleoptiles at various stages of germination.

RESULTS AND DISCUSSION

Oxidative properties of the mitochondrial fractions

Mitochondria isolated by the 'rapid' technique from wheat coleoptiles are probably contaminated by other cell fractions, but this method allows the best preservation of mitochondrial integrity. With this technique which consisted of submitting cell homogenates to high acceleration for a short time, the ADP:O values with α ketoglutarate (αKGA) as a substrate although weaker than the theoretical ones, were not too far removed from values indicated by other workers [3, 4]. Interestingly, the ADP:O values (Fig. 1) decreased during germination for each genotype. A striking difference is observed between F_1 and MSC on one hand, and C and WRH on the other. The mitochondria of F_1 have the highest ADP:O values at all stages of germination; the difference is very large after 5 days growth. It is of interest that the male-sterile Capitole presents ADP:O values higher than those of the fertile one (which differs only in the cytoplasm). The O_2 comsumption (expressed in μAtom O/min/mg mitochondrial protein) is weaker in the F_1 (37.7) and WRH (25.2) than in MSC (72.8) and C (59.9). The F_1 hybrid phosphorylating system seems to be more efficient, although the ADP:O of the hybrid are always higher than in the other genotypes, even the female parent, and the O_2 consumption is weaker, like the male parent. All these data are in agreement with a higher

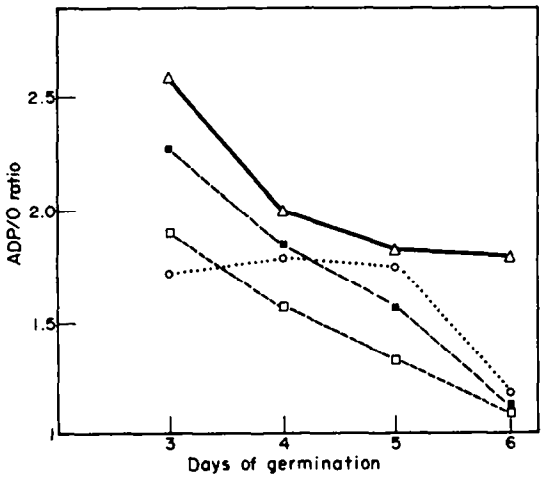

Fig. 1. Oxidative properties of mitochondrial fractions isolated from wheat coleoptiles. The ADP:O ratios, measured with α KGA as substrate, are the mean of 8 determinations. □ Capitole, ■ MSC (female parent), △ F_1 = MSC × WRH ○ WRH (male parent).

efficiency of the mitochondria isolated from the scutellum of maize [5] and wheat [6, 7] in the hybrid than in the parents. However all these observations were restricted to seedlings at a definite stage of germination (2.5 days) and to the 2 parents and hybrid. The present data, obtained on wheat seedlings extend these observations, since changes during germination and the isogenic line on *T. aestivum* (fertile) cytoplasm of the female parent were also analyzed. We can therefore see the typical influence of cytoplasmic male-sterility on mitochondria.

Protein composition

Electrophoretic separations performed on mitochondrial fractions revealed the presence of more than 24 bands for each preparation. Fig. 2 shows that mitochondrial preparations obtained from the seedlings of all 4 genotypes 3 days after germination do not strikingly differ from each other. The main bands designated by their MW $\times 10^{-3}$ namely 21.5, 30, 39, 48, 67, 94 were always found. In addition a band 54 exists in MSC, C, and in WRH, while in the F_1 hybrid there are two other bands (52 and 57). During germination, changes were noticed: 54 disappears and was replaced by 52 and 57 bands in the mitochondria of WRH, Capitole and MSC.

To follow the changes conveniently, the mitochondrial polypeptides were classified in 3 groups differeing by their MW: group 1 comprises polypeptides from 94 to 62, group 2 from 62 to 45 and group 3 from 35 to 24. It may be noticed that during germination a decrease in the relative amounts of proteins of low MW (group 1) (Table 1). This change was noticed for all the genotypes but not at the same time. Again the F_1 hybrid shows a more rapid change. These data are in agreement with the studies of the oxidative properties, since evolution of the mitochondria differs in F_1 hybrid and in the parents.

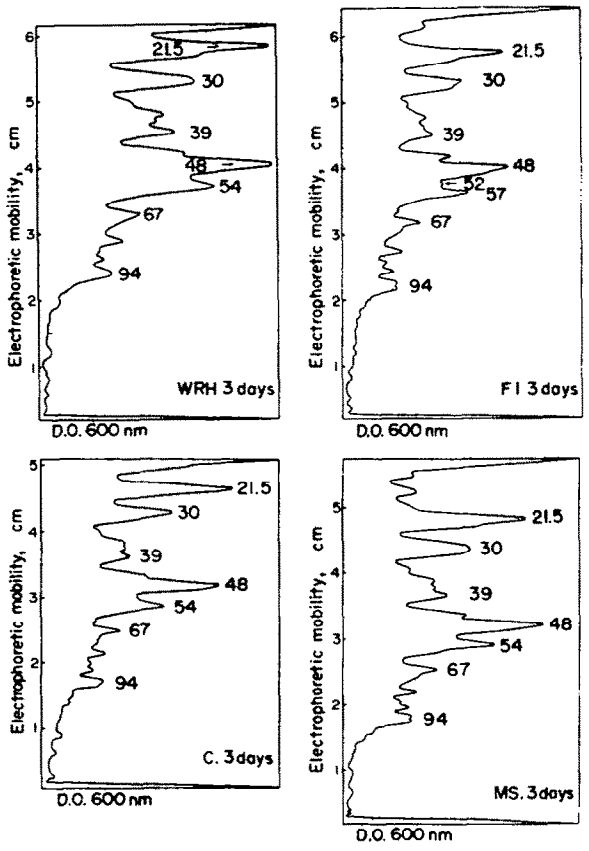

Fig. 2. Electrophoretic patterns of polypeptides of mitochondrial fractions isolated from wheat coleoptiles after 3 days of germination. The number indicated at the top of major-bands correspond to the MW in kilodaltons.

Lipid constituents

The fatty acids play an important role in membranes probably controlling physiological activity [8, 9, 10, 12]. It was of interest to study the total fatty acids of wheat mitochondria. After 3 days of germination, a net difference exists in the fatty acid contents of the mitochondrial pellets isolated from the 4 seedling populations (Fig. 3). MSC is richer in fatty acids than its fertile counterpart, thus richness seems to be a chatacteristic of the male-sterile cytoplasm. During germination a general decrease, greatest in MSC was noticed, and the amount reached homogeneous values exepted for MSC. Relative to the amount of proteins, the amount of fatty acids is almost constant, indicating a strict balance between the biosynthesis and the degradation of fatty acids during the life of the cell. This is in agreement with the results of other workers [9]. The fatty acid composition is qualitatively the same in the mitochondria of the 4 genotypes, but quantitative variations characterize each day of germination.

The main unsaturated fatty acids are oleic, linoleic linolenic acids, and the saturated ones palmitic and stearic acids. Palmitic acid is abundant in the F_1 hybrid and in MSC, and decreases after 3 days of germination. Interestingly linoleic acid, the major fatty acid of wheat mitochondria, undergoes striking variations. After 3 days of germination, linoleate is particularly abundant; its amount strongly decreases during germination for 5 days, and then increases again. These variations of linoleate,

Table 1. Polypeptide composition of mitochondrial fractions isolated from 4 genotypes of wheat coleoptiles. Total protein weight is indicated for each group of polypeptides, group 1: polypeptides from 94000 to 62000; group 2: from 62000 to 45000; group 3: from 45000 to 24000. (Arbitrary units corresponding to triangulation of major peaks).

Genotype	Days of germination	Total proteins: Group 1	Group 2	Group 3
Capitole	3	16	28	29
	4	20	36	29
	5	23	36	26
	6	23	46	22
MS Capitole (female parent)	3	19	26	39
	4	21	31	36
	5	26	30	34
	6	30	36	25
WRH (male parent)	3	17	29	46
	4	24	31	34
	5	21	30	34
	6	28	38	30
F_1 = MSC × WRH	3	18	37	35
	4	27	36	34
	5	32	38	30
	6	31	40	23

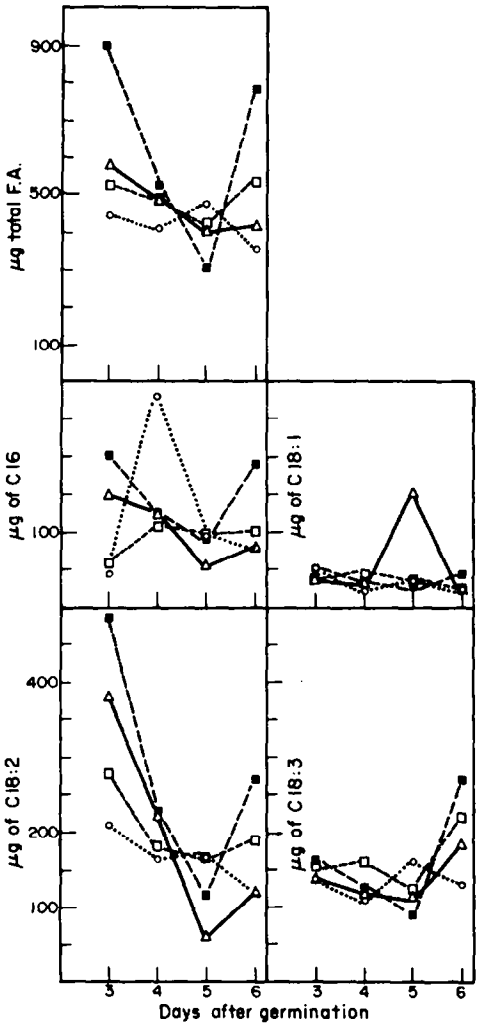

Fig. 3. Variations of fatty acid composition of mitochondrial fractions isolated from wheat coleoptiles during germination. The amounts of the total fatty acids (total FA), of palmitic (C_{16}), oleic ($C_{18:1}$), linoleic ($C_{18:2}$) and linolenic ($C_{18:3}$) acids are indicated in μg. □ Capitole, ■ MSC (female parent), △ F_1 = MSC × WRH, ○ WRH (male parent).

are in a major part responsible for the changes in the total amount of fatty acids, since the amounts of the other fatty acids show only slight variations during germination. These variations of linoleic acid content may be explained by changes in the rhythm of biosynthesis and of the degradation of this fatty acid. It is known that the biosynthesis of linoleic acid is strictly regulated in the life of the plant cell. The oleoyl CoA desaturase, a key enzyme in the biosynthesis of linoleic acid, is only active during short periods in the life of the cell corresponding to the formation of new biomembranes [9].

The F_1 hybrid is characterised by an accumulation of stearic acid 5 days after germination. One is tempted to correlate the changes in physiological activity with the changes in fatty acid content. ADP:O values change during germination in parallel with the quantity of linoleate, and MSC and F_1 which have the higher ADP:O values also have the higher linoleate content. The peak of stearic acid at 5 days in the F_1 hybrid can also be correlated with its higher efficiency at the same time.

EXPERIMENTAL

Plant material. The experiments were performed on the following material. The two inbred lines: WRH 69002 and Capitole: a cytoplasmic male-sterile form of Capitole (MSC) and a F_1 hybrid from the crossing of MSC with WRH 69002. The seeds were humidified with $CaCl_2$ (10^{-4}M), on paper sheets in plastic trays. After 3, 4, 5 and 6 days at a temp. of 23°, the aerial part of the seedlings, (except scutellum), called coleoptile in the text, was removed. Coleoptiles (6 g) were immediately cooled to 0°.

Isolation of mitochondria. Tissues were ground with sand, in sucrose 0.5 M, Pi buffer 67 mM, EDTA 10^{-3} M, BSA (0.1%) and cysteine chloride (0.5%), pH 7.4. The grinding medium is a modification of sols given in ref. [3, 11, 13]. The homogenate was filtered through 4 nylon sheets (48 mesh). After centrifuging at 1000 *g* for 15 sec, the supernatant was centrifuged at 40 000 *g* for 2 min; the mitochondrial pellets were carefully resuspended in 1 ml of grinding medium. The protein content of mitochondrial suspension was determined by the method of ref. [14].

Oxidative properties. The oxidative capacities of mitochondrial prepns were determined by the amperometric method of ref. [15]. The medium was mannitol 0.3 M, KCl 10 mM, $MgCl_2$ 5 mM, HCl 10 mM, $K_2H_2PO_4$ 10 mM BSA 0.1% (pH 7.5). Mitochondria were introduced in the assay mixture (final vol. 3 ml) at 27°; α-ketoglutarate and malonate (final concs 10 mM and 2mM resp) were added to start the reaction. ADP (final concn of 0.3 M) was introduced after a few min. ADP:O values and QO_2, calculated as described in ref. [15], were determined on 8 different amperometric traces, obtained from 4 different mitochondrial isolations.

Electrophoresis of mitochondrial proteins. The mitochondrial proteins were solubilised and electrophoresed using SDS polyacrylamide gels [16]. Mitochondrial suspensions were mixed with equal vol. of Tris SDS 4%, glycerol 40%, mercaptoethanol 10%, and heated at 100° for 3 min. The solubilised proteins mixed with bromophenol blue, were electrophoresed for 4 hr (4 mA/tube) in gels (7 cm long × 0.7 cm i.d.) [16]. The proteins in the gels were detected by Amido black (0.4%) staining, densitometric readings at 600 nm and triangulation of the peaks allowed a determination of the relative importance of the bands. A linear relationship was established between the log of MW of the proteins and their mobility in the gels. With 7.5% acrylamide, the linearity was correct for polypeptides from 94 to 20 × 10^3, verified by using proteins of known MW: α, β, β′ subunits of RNA polymerase (respectively 39 000, 155 000 and 165 000), BSA (68 000), trypsin inhibitor (21 500).

Analysis of fatty acids. The fatty acids of the pellet were extracted and methylated [17]. The Me esters were analysed by FID GLC, using a solumn (6 mm × 5.3 m) containing 20% butane-diol-succinate on silanised Chromosorb W (60–80 mesh), isothermal (190°) with Na as carrier (20 ml/min). The introduction of a known amount of Me heptadecanoate in the Me ester soln allowed the determination of the amounts of each fatty acid.

Acknowledgements—The authors are much indebted to Prof. Y. Demarly and P. Mazliak for critical reading of the manuscript. The skilful assistance of M. Willemin is also appreciated.

REFERENCES

1. Demarly, Y. (1972) *Ann. Amelioration Plantes* **22**, 143.
2. Demarly, Y. (1974) *Agron. Trop.* **XXIX**, 871.
3. Ikuma, H. and Bonner, W. D. (1967) *Plant Physiol.* **42**, 67.
4. Lance, C. (1971) *Physiol. Veg.* **9**, 259.
5. McDaniel, R. G. and Sarkissian, I. V. (1970) *Physiol. Plantarum* **23**, 335.
6. Srivastava, H. K. (1970) Doctoral Dissertation, Texas A & M University.
7. Sage, G. C. M. and Hobson, G. E. (1973) *Euphytica* **22**, 61.

8. Chapman, D. and Wallach, D. F. H. (1968) in *Biological Membranes* (Chapman, D. ed.)p. 125. Academic Press, New York.
9. Mazliak, P. (1973) *Ann. Rev. Plant Physiol.* **24**, 287.
10. Jolliot, A. (1972) *Année Biologique* **11**, 351.
11. Hulme, A. C., Jones, J. D. and Woltorton, L. S. C. (1964) *Phytochemistry* **3**, 173.
12. Coleman, R. (1973) *Biochim. Biophys. Acta* **300**, 1.
13. Sarkissian, I. V. and Srivastava, H. K. (1968) *Plant Physiol.* **43**, 1406.
14. Lowry, O. H., Rosebrough, H. L., Farr, S. L. and Randall, J. R. (1951) *J. Biol. Chem.* **193**, 265.
15. Chance, B. and William, G. R. (1955) *Nature* **227**, 680.
16. Laemmli, U. K. (1970) *Nature* **227**, 680.
17. Metcalfe, L. D., Schmitz, A. A. and Pelika, J. R. (1966) *Anal. Chem.* **38**, 514.
18. Lechevallier, D. (1966) *C. R. Acad. Sci. Paris* Série D **263**, 1849.