COMPARATIVE STUDIES OF FUNCTIONS OF MITOCHONDRIA FROM A POLYPLOID SERIES OF WHEAT[1]

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Received February 2, 1970

COMMON wheat (*Triticum aestivum*) is adapted widely because of stabilization of favorable combinations of genes from its progenitors through polyploidy. The concept that the hexaploid wheat involves three genomes, A, B, and D is based largely on evidence of chromosome pairing affinities in interspecific F_1 hybrids and on gross morphological and karyotypic comparisons (SARKAR and STEBBINS 1956; SEARS and OKAMOTO 1958; RILEY and CHAPMAN 1966). A more direct approach for estimating phylogenetic affinities at the species level has been advanced recently through electrophoresis of proteins (JOHNSON, BARNHART and HALL 1967; SING and BREWER 1969). The general conclusion drawn from these studies was that duplicated loci of polyploid species specify heteromultimers (polypeptides) which may possess adaptive functional advantages over two functional homomultimers of their diploid progenitors.

Organelles other than the nucleus are important in inheritance and in the direction of cell function (WILKIE 1964; BEALE 1966; WAGNER 1969). GRANICK and GIBOR (1967) have presented information showing that chloroplasts, mitochondria, and centrioles of the eukaryotic organisms possess heritable systems independent of the nuclear system. CASPARI (1956) suggested that mitochondria could be important in maintaining intracellular homeostasis and that increased homeostasis of mitochondrial constitution could provide a physiological basis for heterozygote superiority. We have demonstrated the role of mitochondria in the manifestation of high efficiency, heterosis, and homeostasis in wheat (SARKISSIAN and SRIVASTAVA 1969).

Few comparisons of cytoplasmic characters of polyploid series of wheat have been made thus far, but a positive correlation of mitochondrial function and plasmon heterogeneity has been observed in several crosses of wheat (SRIVASTAVA, SARKISSIAN and SHANDS 1969). To extend that knowledge we chose in the present study to characterize and evaluate some wheat genomes with regard to mitochondrial function, and to learn whether differences between species, genera, and higher taxonomic units exist in terms of mitochondrial functions.

MATERIALS AND METHODS

Representatives of hexaploid, tetraploid, and diploid species of Triticum as well as their diploid

[1] This investigation was supported in part by DeKalb AgResearch, Inc.

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and tetraploid Aegilops relatives were used. The species representing each of the members of the polyploid series (designated as Genome group) are given below (cf. LILIENFELD 1951).

Level of polyploidy	Genome group	Species
Diploid	AA	*Triticum monococcum*
		Triticum boeoticum
Tetraploid	AABB	*Triticum dicoccum*
		Triticum durum
		Triticum turgidum
		Triticum polonicum
Hexaploid	AABBDD	*Triticum aestivum*
		Triticum spelta
		Triticum vavilovii
		Triticum sphaerococcum
		Triticum macha
Diploid	SS	*Aegilops bicornis*
	CC	*Aegilops caudata*
Tetraploid	C^uC^uMM	*Aegilops ovata*
	C^uC^uCC	*Aegilops triuncialis*
	C^uC^uSS	*Aegilops variabilis*

Preparation of mitochondria: Shoots (sprouts) of 2–3 day old seedlings which had been grown at 27°C in the dark on moistened paper toweling served as sources of mitochondria. Techniques of measurement of respiration were identical with those described earlier (SARKISSIAN and SRIVASTAVA 1968). Techniques of isolation of mitochondria had been fully described (SARKISSIAN and SRIVASTAVA 1968, 1969, 1970). In essence, the entire procedure takes only 10 minutes and we found that mitochondria when isolated so rapidly are not damaged (as witnessed by their activity) and are not as variable as mitochondria isolated by conventional techniques (SARKISSIAN and SRIVASTAVA 1968). Oxidation rates (State 3 and State 4) were measured polarographically at 27°C. Respiratory control (RC) values were calculated as the ratio of State 3 to State 4 rates (CHANCE and WILLIAMS 1956). ADP : O ratios were determined from the amount of oxygen utilized during State 3 (μatoms oxygen) responding to a known amount of added ADP* (μmol).

Measurement of adenosine triphosphatase and cytochrome c oxidase activity: For measurement of ATPase activity, mitochondria were isolated rapidly but in 0.45 M sucrose, pH 7.2. Reactions were carried out for 10 min at 37°C. Reaction mixtures contained in 3 ml, pH 7.3, 5 mM KCl, 3 mM $MgSO_4$, 0.45 M sucrose, 20 mM Tris-HCl, 1 mM ATP and about 0.27 mg of mitochondrial protein. Reactions were stopped by withdrawing a 2 ml aliquot from each sample and adding it immediately to 2 ml of cold 10% TCA. Difference in P_i released by a sample and its zero time control was used as a measure of enzyme activity of each sample. For measurement of cytochrome c oxidase activity, mitochondria were prepared in the standard grinding buffer used in isolation of mitochondria for oxidation of NAD-linked substrates (SARKISSIAN and SRIVISTAVA 1969). Thirty ml of 1.7×10^{-5} M cytochrome c in 0.03 M phosphate buffer, pH 7.4, were reduced in an Erlenmeyer flask by addition of 0.1 ml of freshly prepared solution of sodium hydrosulfite (1.2 M) followed by vigorous stirring for 2 min. One ml of the reduced cytochrome c solution and 0.01 or 0.02 ml of buffered mitochondrial suspension containing 15 to 20 μg of protein were placed in a cuvette. The reactants were mixed and readings were taken at 550 mμ (COOPERSTEIN and LAZAROW 1951). Reactions were run at 30°C and the rate of oxidation of cytochrome c was constant during at least two minutes.

* Abbreviations: ADP, adenosine 5′-diphosphate; ATPase, adenosine triphosphatase; NAD, nicotinamide adenine dinucleotide; ATP, adenosine 5′-triphosphate.

Mitochondrial nitrogen was estimated from mitochondrial protein determined by a modified method of LOWRY and coworkers (1951) with bovine serum albumin as a standard.

RESULTS

Respiratory activities of mitochondria of wheat species utilizing α-ketoglutarate as substrate are presented in Table 1. The diploid species, *T. monococcum* and *T. boeoticum*, exhibited low oxidative and phosphorylative activities as compared with the tetraploid and the hexaploid species. All the tetraploid species were approximately intermediate between the diploid and the hexaploid species as witnessed by their State 3 oxidation, RC and ADP:O ratios. No consistent differences were observed between the diploid and the tetraploid species with regard to State 4 oxidation. However, the hexaploid species showed a relatively greater rate of State 4 oxidation. These results suggested the possibility that wheat polyploids may differ in mitochondrial function. This proposal is further strengthened by the data included in the bottom of Table 1 concerning Aegilops species. It is of interest that the diploid species of Aegilops exhibited lower oxidative and phosphorylative activities than did the tetraploid species as witnessed by their State 3 oxidation and RC and ADP:O ratios. Once again, no marked differences between these species existed with respect to State 4 oxidation. It should be noted at this point that our criteria of evaluation of the respiratory properties of these species were State 3 oxidation, RC and ADP:O ratios.

TABLE 1

Oxidative and phosphorylative activities of mitochondria from polyploid series of Triticum and Aegilops

Source of mitochondria	O_2 uptake (μ moles O_2/min/mg N) State 3	State 4	RC ratio	ADP:O ratio
Triticum monococcum (2)*	24.0 ± 2.2	9.6 ± 0.6	2.5	2.4 ± 0.10
Triticum boeoticum (2)	26.0 ± 1.4	9.8 ± 0.5	2.6	2.3 ± 0.12
Triticum dicoccum (4)	30.1 ± 2.6	9.1 ± 1.2	3.3	3.2 ± 0.2
Triticum durum (4)	28.4 ± 3.1	8.9 ± 1.1	3.2	3.1 ± 0.15
Triticum turgidum (4)	30.6 ± 2.4	9.0 ± 1.3	3.4	3.2 ± 0.17
Triticum polonicum (4)	27.5 ± 3.4	8.6 ± 1.4	3.2	3.3 ± 0.14
Triticum macha (6)	47.8 ± 3.9	13.3 ± 1.5	3.6	3.5 ± 0.10
Triticum spelta (6)	43.5 ± 4.2	11.7 ± 1.4	3.7	3.6 ± 0.12
Triticum vavilovii (6)	40.2 ± 4.7	11.2 ± 1.6	3.6	3.5 ± 0.20
Triticum sphaerococcum (6)	39.7 ± 6.4	10.2 ± 2.1	3.9	3.4 ± 0.21
Triticum aestivum (6)	45.8 ± 6.2	10.4 ± 1.9	4.4	3.8 ± 0.19
Aegilops bicornis (2)	21.4 ± 1.9	10.7 ± 0.8	2.0	2.1 ± 0.10
Aegilops caudata (2)	23.5 ± 1.8	10.2 ± 1.1	2.3	2.1 ± 0.12
Aegilops triunciales (4)	26.2 ± 1.2	9.5 ± 1.4	2.7	2.6 ± 0.14
Aegilops ovata (4)	28.7 ± 2.1	9.8 ± 1.6	2.9	2.5 ± 0.20
Aegilops variabilis (4)	26.1 ± 1.6	8.7 ± 1.8	3.0	2.4 ± 0.15

α-ketoglutarate was used as substrate. Data are averages of 6 separate experiments ± SE

* The number in parentheses refers to the level of ploidy.

TABLE 2

Mitochondrial adenosine triphosphatase activity from polyploid series of Triticum and Aegilops

Source of ATPase	Specific activity (μ moles P_i released/mg protein/hr)
Triticum monococcum (2)*	78.5 ± 6.4
Triticum boeoticum (2)	76.4 ± 5.4
Triticum dicoccum (4)	118.4 ± 7.5
Triticum durum (4)	110.5 ± 8.2
Triticum turgidum (4)	120.7 ± 9.1
Triticum polonicum (4)	105.0 ± 8.7
Triticum macha (6)	137.3 ± 11.2
Triticum spelta (6)	142.5 ± 12.4
Triticum vavilovii (6)	130.5 ± 13.4
Triticum sphaerococcum (6)	134.2 ± 10.4
Triticum aestivum (6)	147.5 ± 12.7
Aegilops bicornis (2)	65.0 ± 4.8
Aegilops caudata (2)	68.0 ± 5.2
Aegilops triunciales (4)	82.4 ± 6.4
Aegilops ovata (4)	89.5 ± 6.2
Aegilops variabilis (4)	85.0 ± 7.6

Data are averages of 6 separate experiments ± SE.
* The number in parentheses refers to the level of ploidy.

To further check the differences among the polyploid series with regard to mitochondrial energetics, ATPase activity was assayed. The data in Table 1 had revealed the relative efficiencies of the ADP:O ratio which is equivalent to the P:O ratio or the number of molecules of ATP synthesized per μatom of oxygen used in respiration. Activity of ATPase would be indicative of the rate of release of energy from ATP (Table 2). The diploid species of wheat was lower in activity than the tetraploid which in turn was lower than the hexaploid. There was also a marked difference between the diploid and the tetraploid species of Aegilops in ATPase activity, the tetraploid species expressing higher specific activity than did the diploid species (Table 2). Also, the difference between the diploid species of wheat and Aegilops (*T. monococcum* and *T. boeoticum* vs. *A. bicornis* and *A. caudata*) was not significant with respect to ATPase activity.

Activity of cytochrome c oxidase from wheat and Aegilops is summarized in Table 3. The diploid species of wheat had lower specific activity as compared with the tetraploid and the hexaploid species. As was the case with ATPase activity, the hexaploid wheats showed highest cytochrome c oxidase activity. Again, the diploid species of Aegilops possessed the lowest activity. The data in Table 3 are of interest because they summarize specific activities of cytochrome oxidase from the polyploid series. While further kinetic analysis would have been more informative, difficulty in obtaining sufficient amounts of plant tissue for purification of the enzyme precluded this type of analysis. We have prepared a highly purified cytochrome oxidase from a hexaploid strain and a hexaploid

TABLE 3

Cytochrome c oxidase activity from a polyploid series of Triticum and Aegilops

Source of Cytochrome c oxidase	Specific activity (ΔOD at 550 mμ/min/mg protein)
Triticum monococcum (2)*	6.2 ± 1.2
Triticum boeoticum (2)	5.7 ± 1.4
Triticum dicoccum (4)	8.9 ± 1.0
Triticum durum (4)	10.2 ± 1.3
Triticum turgidum (4)	9.8 ± 0.8
Triticum polonicum (4)	9.4 ± 0.7
Triticum macha (6)	11.2 ± 1.2
Triticum spelta (6)	13.3 ± 2.0
Triticum vavilovii (6)	12.4 ± 1.8
Triticum sphaerococcum (6)	11.8 ± 0.8
Triticum aestivum (6)	13.4 ± 2.1
Aegilops bicornis (2)	5.4 ± 1.0
Aegilops caudata (2)	5.2 ± 1.5
Aegilops triunciales (4)	7.9 ± 1.3
Aegilops ovata (4)	8.2 ± 1.1
Aegilops variabilis (4)	7.6 ± 1.2

Data are averages of 6 separate experiments ± SE.
* The number in parentheses refers to the level of ploidy.

hybrid and noted striking differences in kinetics of the enzyme (SARKISSIAN and SRIVASTAVA, in preparation). This type of analysis could not be carried out with the polyploid series. It is important to point out that the differences which we have observed are, in all probability, real differences and not the result of variations in mitochondrial damages. Consistency in behavior of isolated mitochondria in our hands (SARKISSIAN and SRIVASTAVA 1968, 1969, 1970) compels us to make this statement.

DISCUSSION

The hexaploid wheats exhibited superior mitochondrial function with respect to all the parameters used for comparison. Such observations would mean that mitochondria from the hexaploid species, including the common wheat, possess relatively efficient oxidative phosphorylation, adenosine triphosphatase and cytochrome c oxidase activities. The increased efficiency of ATP synthesis, as judged by ADP:O ratios, and the release of energy of ATP, as judged by adenosine triphosphatase activity, may provide the hexaploid wheat species a biochemical advantage and may make it adaptable to variable environments. The precise mechanism of the superior mitochondrial functions in the hexaploid wheat species is unknown. We think however, that these species have polymorphic mitochondria, the polymorphism having arisen during the course of evolution of wheat. The vigor of mitochondrial functions manifested by the hexaploid species may be the result of complementation (MCDANIEL and SARKISSIAN 1966) between

different types of mitochondria present in these species. The occurrence of polymorphic mitochondria in heterotic hybrids of maize (SARKISSIAN and MCDANIEL 1967) and wheat (unpublished results) has been demonstrated. These observations are of much interest because they not only demonstrated heterogeneity of mitochondria but also suggested that different mitochondria, like different allelic genes, may produce "heterozygous" combinations in hybrids which are superior to their inbred parents. Our findings show that hexaploid wheat species excel their progenitors in mitochondrial functions. This superiority could be the result of polymorphic mitochondria present in the hexaploid. Greater protein diversity in Triticum than in Aegilops relatives as well as interspecific differences in the average number of isozymes of Triticum (SING and BREWER 1969) have suggested a selective advantage of polyploid wheats over their progenitors.

From results of studies of mitochondrial systems from various species of Triticum and Aegilops, it is obvious that mitochondrial variability is reflected in the levels of enzymatic activity. There seemed to be a positive relationship between the levels of enzymatic activity of mitochondria and the levels of polyploidy of the species. The tetraploid species were found to be intermediate between the diploid and the hexaploid species of Triticum with regard to all the criteria used for comparison. Significant differences were also observed between species of Aegilops and Triticum. These findings support the view that differences among species, genera, and higher taxonomic orders of wheats observed in the present experiments result from heterogeneity of mitochondria. We think that efficiency of mitochondrial function is directly proportional to the degree of polymorphism of mitochondria. Thus we conclude that behavior of mitochondria in a polyploid series of wheat may exhibit complementation which reflects mitochondrial heterogeneity. While polymorphism (as judged by mitochondrial activity) may confer adaptive advantages to a species from the point of view of distribution and agronomic value, at this writing we cannot discuss chromosome dosage and its possible relationship to the degree of mitochondrial polymorphism. The possibility that in wheats chromosome dosage may be directly related to mitochondrial polymorphism and to adaptive superiority is attractive, but it demands that we learn more about the biogenesis of mitochondria and its control by chromosomal genes before additional statements are made.

SUMMARY

Significant differences were found among mitochondria of diploid, tetraploid, and hexaploid species of Triticum and Aegilops with regard to oxidative phosphorylation, adenosine triphosphatase and cytochrome c oxidase activities. The hexaploid wheat species possessed relatively efficient α-ketoglutarate dehydrogenase, adenosine triphosphatase, and cytochrome c oxidase systems. The results suggest that the widely adapted hexaploid wheats possess more efficient mechanisms for ATP synthesis as well as for the release of energy from ATP than do the diploid and tetraploid species of Triticum and Aegilops. The findings were discussed with the point of view that mitochondria are important in providing a

portion of the overall biochemical adaptive advantage to the hexaploid wheat species.

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