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Cold induced changes in the morphology of mitochondria in coleoptiles of corn of different frost-resistance

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Biochemical studies on the hardiness of cereals suggest that there exists a certain relation in plants between the ability of maintaining a higher biochemical activity of the mitochondria in the winter period, and a greater resistance to frost. This relation is indicated both by investigations on the respiration intensity of hibernating cereal leaves (Semikhatova 1962; Borzhkovskaya and Usova 1968) and studies on the energetic efficiency of the mitochondria isolated from these plants and expressed by the P:O ratio (Borzhkovskaya and Usova 1968). It seemed, therefore, useful to check, whether in plants of different hardiness there are any differences in the morphological changes in the mitochondria caused by cold.

MATERIAL AND METHODS

The observations were carried out on material which had been the object of physiological and biochemical studies of the above named authors (Borzhkovskaya and Usova l. c.). This material consisted of seedlings of the following plants: *Agropyron glaucum* (genome BDX, 2n = 42), *Triticum aestivum* var. *lutescens*, cv. Lutescens 329 (genome ABD, 2n = 42), and of their hybrids which are incomplete amphidiploids (2n = 56), denoted by the symbols TAN 822 (genome ABD_TD_A) and TAN 829 (genome ABD_TX_A), and also of two other winter wheat varieties: *Triticum aestivum* L. var. *velutinum* cv. Ulyanovka and *Triticum aestivum* var. *erythrospermum* Körn. cv. Kooperatorka. From among the above mentioned plants *A. glaucum* exhibits the highest resistance to cold, and TAN 829 which inherits the phenotypic features from *Agropyron* is similar to it in this respect. The second hybrid TAN 822 is moderately resistant to frost like its maternal form Lutescens 329 (Khvostova et al. 1963). Cv. Kooperatorka is but little resistant to cold, whereas cv. Ulyanovka is considered as the most hardy wheat in Siberia.

Since the cytological literature fails to give any detailed data on the behaviour of mitochondria within cells of cereals subjected to low temperatures, preliminary studies had to be carried out in order to establish the thermic conditions which would elicit morphological changes detectable in the light microscope and to become acquainted with the general regularities occurring in these changes. These preliminary observations were made on two kinds of plants which markedly differed in hardiness, i.e. *A. glaucum* and the winter wheat cv. Lutescens 392.

Living cells of the coleoptile epidermis were investigated. Seedlings were cultivated on filter paper moistened with tap water on Petri dishes kept in the dark in a thermostat at +25°C. Seedlings were taken for study when their coleoptile were 25 mm long. Plant preparations just taken out of the thermostat served as control material. They were inspected in tap water or in silicon oil (Crossmon 1967) at room temperature (20—25°C). The coleoptile epidermis of the chilled plants was observed in silicon oil only both at temperature lowered to the corresponding experimental value and at room temperature during defrosting.

The epidermis of the apical coleoptile part (1.5—2.0 mm from the apex) was inspected since it proved to be particularly convenient for observations owing to the fact that in control conditions almost uniform mitochondria occur in it. They are spherical, but a few have the shape of short rods (Plate II, photo 1). This feature was characteristic of *A. glaucum*, cv. Lutescens 329 and for the remaining material studied when cultivated in the same conditions.

RESULTS OF OBSERVATION

Preliminary experiments proved that changes in shape and size of the mitochondria detectable in the light microscope occur between 0°C and —8°C. Thus, systematic observations were performed within this temperature range. Low temperatures were achieved with a ±0.1°C accuracy in a Feutron barochamber in which the seedlings were placed in Petri dishes.

The reaction of the organism to cold is known to depend not only on the lowering of the temperature, but also on the time of exposure of the plant, and the rate at which the organism is chilled to the given temperature (Aleksandrov 1964; Rottenberg 1968, and others). In order to obtain a fuller picture of the mitochondrial changes, the experiments were made in three variants: 1) the temperature was changed suddenly from +25° to 0°, —2°, —3°, —4°, —5°, —6°, —7° and —8°C, respectively; 2) the temperature was lowered stepwise by transferring the plants from +25° to 0°C for 24 hrs., then for the next 24 hrs. from 0°C to —2°C, and so on; 3) in keeping with Tumanov's theory (Tumanov 1960; Tumanov and Trunova 1963 and others) claiming that the increase of hardiness of plants occurs in the autumn-winter season in two steps (step I at temperatures close to 0°C, step II at moderate temperatures below 0°C), the seedlings of the plants under investigation were subjected to a two-step adaptation: for 5—7 days at 0°C (step I), and also for 5—7 days at —4°C (step II). After this treatment the plants were chilled for 24 hrs. at —8°C.

The results of the experiments demonstrated that the shape and size of the mitochondria change independently of the way in which the plants are chilled (Fig. 1 A, B). For temperatures from 0°C to —2°C the appearance of a large number of elongated mitochondria (1.2 to 3.0 μ) which do not occur in control conditions and a decrease in the total number of mitochondria in the cell are characteristic. These two findings suggest that the elongated mitochondria originate from sequential

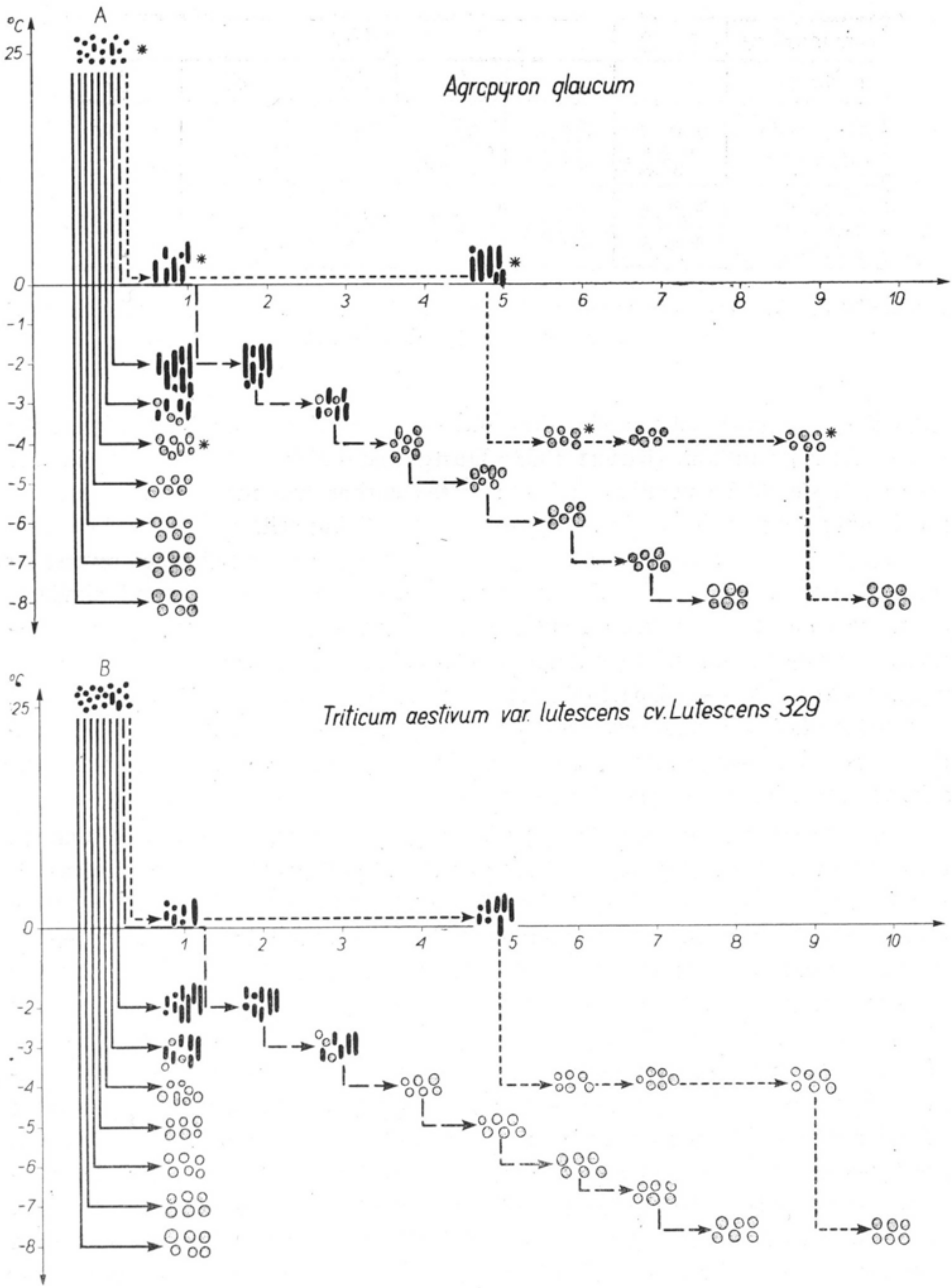


Fig. 1. A and B. Graphic presentation of changes in shape of mitochondria from coleoptile epidermis caused by exposure to temperatures from 0° to −8°C. Comparison of three groups of experiments: 1) → — seedlings transferred directly from +25°C for 24 hrs. to 0°C, −2°C, −3°C and so on; 2) --> — seedlings transferred from + 25° to 0° for 24 hrs. then from 0° to −2°C for 24 hrs. and so on; 3) --> — seedlings adapted to low temperature in two steps: 5 days at 0°C and 5 days at −4°C, and then transferred to −8°C for 24 hrs.

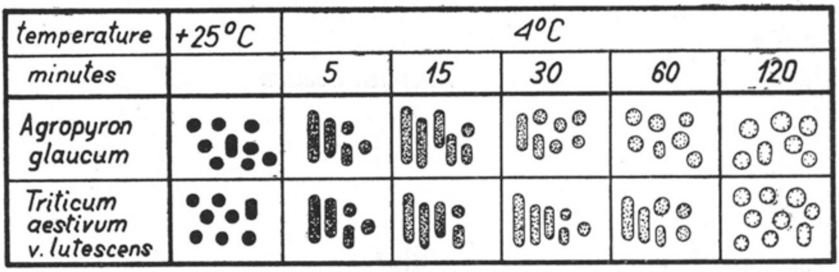


Fig. 2. Changes in shape and size of mitochondria in seedlings subjected to a sudden change of temperature from +25°C to −4°C.

aggregation of spherical mitochondria and short rods. This is consistent with the views of many authors (Buvat 1953; Dangeard 1958; Szabadasz 1966, and others). It should be mentioned here that the author has many times observed the joining (aggregation) of mitochondria in the living cell.

Minus 4° proved to be the threshold temperature causing changes reverse to those described above: the elongated mitochondria disappeared, and similarly as in the control conditions, chiefly spherical forms occurred, however, their diameter increased from 0.4 μ (control) to 0.6—0.7 μ. Not all these organellae were equally swollen. At —3°C, part of the mitochondria had the shape of not very long and not swollen rods, and the others were swollen of oval or spherical shape. Temperatures below —4°C only increased the degree of swelling and rounding of the mitochondria. Their average diameter at —8°C was 0.7—0.9 μ.

The two steps of the morphological changes in the mitochondria, distinct in the experiments with gradual lowering of temperature (Fig. 1), were also noticeable when the plants were transferred suddenly from 25°C to —4°C if observations were made at short intervals. In the first minutes after transferring the plants to —4°C the mitochondria changed to rod-like forms, the number of which, after reaching a maximum, decreased in the further minutes gradually to zero. Then the process of swelling of the spherical mitochondria started (Fig. 2). The mitochondria of the plants first chilled at 0° for 7 days, and then transferred to —4°C behaved in a similar way. Their first reaction to —4°C was an increase in the number of elongated mitochondria up to the complete disappearance of spherical forms, and a still greater elongation as compared to their state at 0°C. Only after 3—4 hrs. did a reversal to the spherical shape and swelling of the mitochondria occur (Fig. 3). A similar two-step reaction was observed by Wrischer and Devidé (1965) in root meristems subjected to respiratory inhibitors and anaerobic conditions.

The changes in the shape of the mitochondria caused by chilling in *Agropyron* and wheat proved reversible. The cells, in which aggregation of mitochondria into long rods had occurred, returned to their initial state 4—5 hrs. after transferring to room temperature.

The swollen mitochondria shrunk to the size of the controls within approx. 20 min. A certain difference was noted in the behaviour of the swollen mitochondria

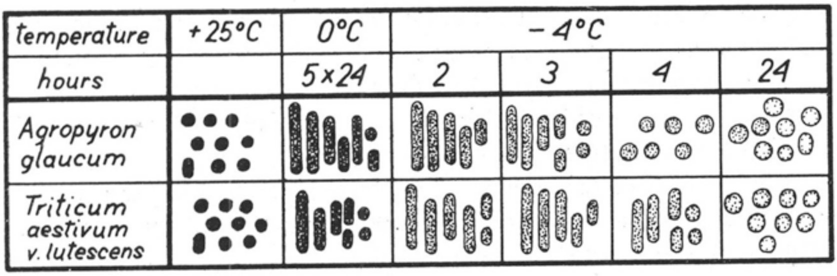


Fig. 3. Changes in shape and size of mitochondria in coleoptiles of seedlings transferred to —4°C after previous adaptation for 5 days at 0°C.

depending on the way in which the plants had been previously chilled. The mitochondria of coleoptiles which had been adapted in step I at 0°C and then transferred for 24 hrs. to —4°C, transformed rapidly into long rods, that is they returned to the shape they had before transfer to —4°C. On the other hand, the mitochondria of plants suddenly cooled from +25°C to —4°C and those of plants which had passed the two-step hardening shrunk without changing their spherical shape (for tentative interpretation of these differences see Kwiatkowska 1970 a).

The in vivo observations discussed were supplemented by investigations in the electron microscope after fixing the material in glutaric aldehyde after Semadanni with postfixing by osmium tetroxide according to Caufield. The investigations were performed on *Agropyron*. The ultrastructure of mitochondria of the control plants was compared with that of plants chilled at 0°C and —4°C according to the variants marked by asterisks in Fig. 1. The chilled plants were fixed without defrosting. The results of the investigations are shown in Plate I. As can be seen, the ultrastructure of mitochondria did not change with the alteration of their shape. The control preparation (Plate I, photo 1) and those chilled for a relatively short time, i.e. 24 hrs. at 0°C (photo 2) and at —4°C (photo 3) exhibited an identical ultrastructure, although the mitochondria greatly differed in shape (cf. in vivo observations, Plate II, photos 1, 2, 3). Only plants chilled at 0° for a week exhibited a certain change in the ultrastructure of their mitochondria consisting in the disappearance of the electron-dense matrix (Plate I, photo 4). The ultrastructure of swollen mitochondria of plants first chilled at 0° for 7 days and transferred to —4°C showed wide changes as compared with the controls, they were almost deprived of the electron-dense matrix and their cristae were greatly reduced (photo 5), both in plants kept at —4°C for 24 hrs. and for 7 days. Thus, in these temperature intervals, the duration of chilling proved more significant for the ultra-structure of mitochondria than the degree of temperature decrease.

As regards the changes in mitochondria observed in living cells, the regularities described here proved to be characteristic both for *Agropyron* and the wheat Lutescens 329, in spite of their belonging to different genera and differing in hardiness. However, it should be added that both these plants are characterized by an ability of adaptation to low temperatures and both originated from the same ecological conditions, so this might have been the reason for the resemblance in the reaction of their mitochondria. It should be remembered that the swelling of mitochondria in *Cichorium intybus* roots, observed by Genevès (1951) occurred already at 0°C, whereas the above described swelling of *Agropyron* and wheat mitochondria took place only at a temperature as low as —4°C.

A deeper analysis of the results, taking into consideration the changes in the number of the particular mitochondrial forms, demonstrated, however, that there exist slight differences between the particular objects examined: after exposure to 0°C for 24 hrs. many more elongated mitochondria formed in *Agropyron* than in wheat. Since the initial pictures were the same in both cases, this would indicate a stronger reaction of mitochondria of the hardy *Agropyron* than of the less resistant wheat.

In this connection it seemed advantageous to check, by comparing the reaction of the mitochondria of all the material investigated to 0°C, whether there is some relation between the appearance of numerous elongated mitochondria in response to this temperature, and the degree of resistance of the plant.

The observations were performed on living material. For quantitative comparison, it was necessary to adopt the method of recording in vivo observations. The mitochondria were classified according to their shape into 4 groups: 1) spherical, 2) short rods, not longer than twice their width, i.e. up to 0.8 μ, 3) moderately long rods, up to 1.2 μ, 4) long rods, the length of which was equal to, or greater than four times their width, i.e. 1.6 μ. Accurate counting of the mitochondria of each class carried by the cytoplasm current was impossible, although their shapes stand out well in these conditions. Therefore, the following rule was adopted in the observations: five cells in one section were carefully inspected and in each the proportion of various mitochondrial forms was quantitatively estimated. The observations were recorded by a corresponding number of "+" marks representing the quantitative relations. In all cases the experiments were repeated four times, each time on three randomly chosen individuals. The results in the particular repetitions were very similar. They lead to the following conclusions: 1) the largest number of elongated mitochondria was found after chilling to 0° in the *Agropyron* epidermis; 2) in all wheats, notwithstanding their degree of hardiness, the elongated mitochondria were much less numerous; spherical ones and short rods are the dominant forms for them; 3) hybrids react to a weaker degree than *Agropyron*, but stronger than wheat; 4) no greater resemblance ot *Agropyron* could be noted in the more hardy TAN 829 than in the less resistant TAN 822 (see Kwiatkowska 1970 b, Fig. 1). It cannot, therefore, be assumed that there is a relation between the number of elongated mitochondria appearing at 0°C, and the degree of hardiness of the plants.

The second difference which could be observed in the behaviour of the mitochondria of chilled *Agropyron* and wheat seedlings was the rapidity with which the changes in shape occurred. For instance, under the influence of a temperature of —4°C, the whole cycle of changes in *Agropyron* — beginning with the elongation of the mitochondria and ending in a new fragmentation and swelling — lasted about 1 hr., whereas in wheat the same process took 2 hrs. (Fig. 2). The difference in

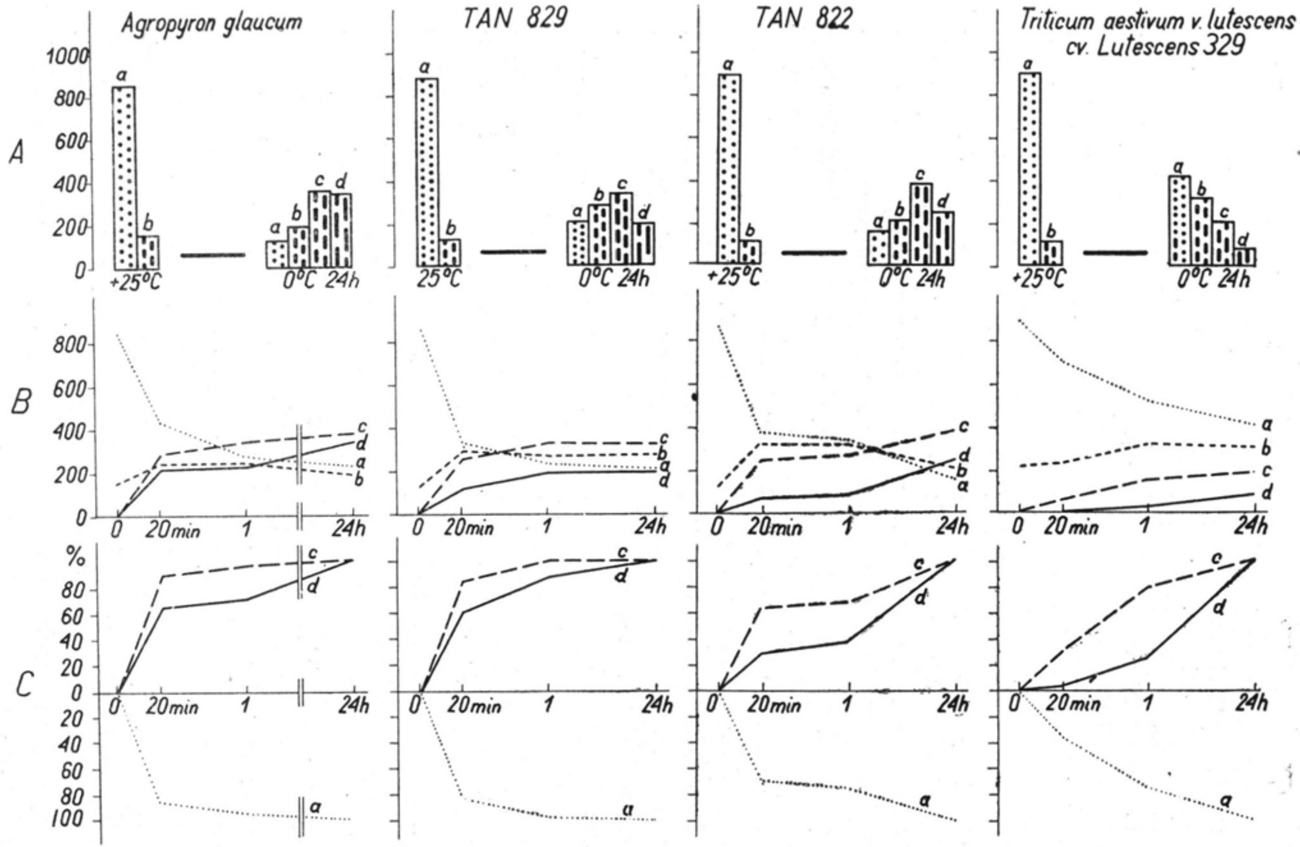


Fig. 4. A. Comparison of occurrence of four types of mitochondria in epidermal cells from coleoptiles kept in darkness at +25°C for 24 hrs., and after 24 hrs. of exposure to 0°C. 1000 mitochondria were counted from 10 plants:

a — spherical forms, *b* — rod-like forms up to 0,8 μ, *c* — rod-like forms about 1.2 μ long, *d* — forms 1.6 μ long and longer.

B. Changes in the proportions of mitochondria of the four types after 20 mins., 1 and 24 hrs. of exposure to 0°C. (Notations as above).

C. Rate of changes in the shape of mitochondria upon exposure to 0°C presented as relative values(for explanation see text).

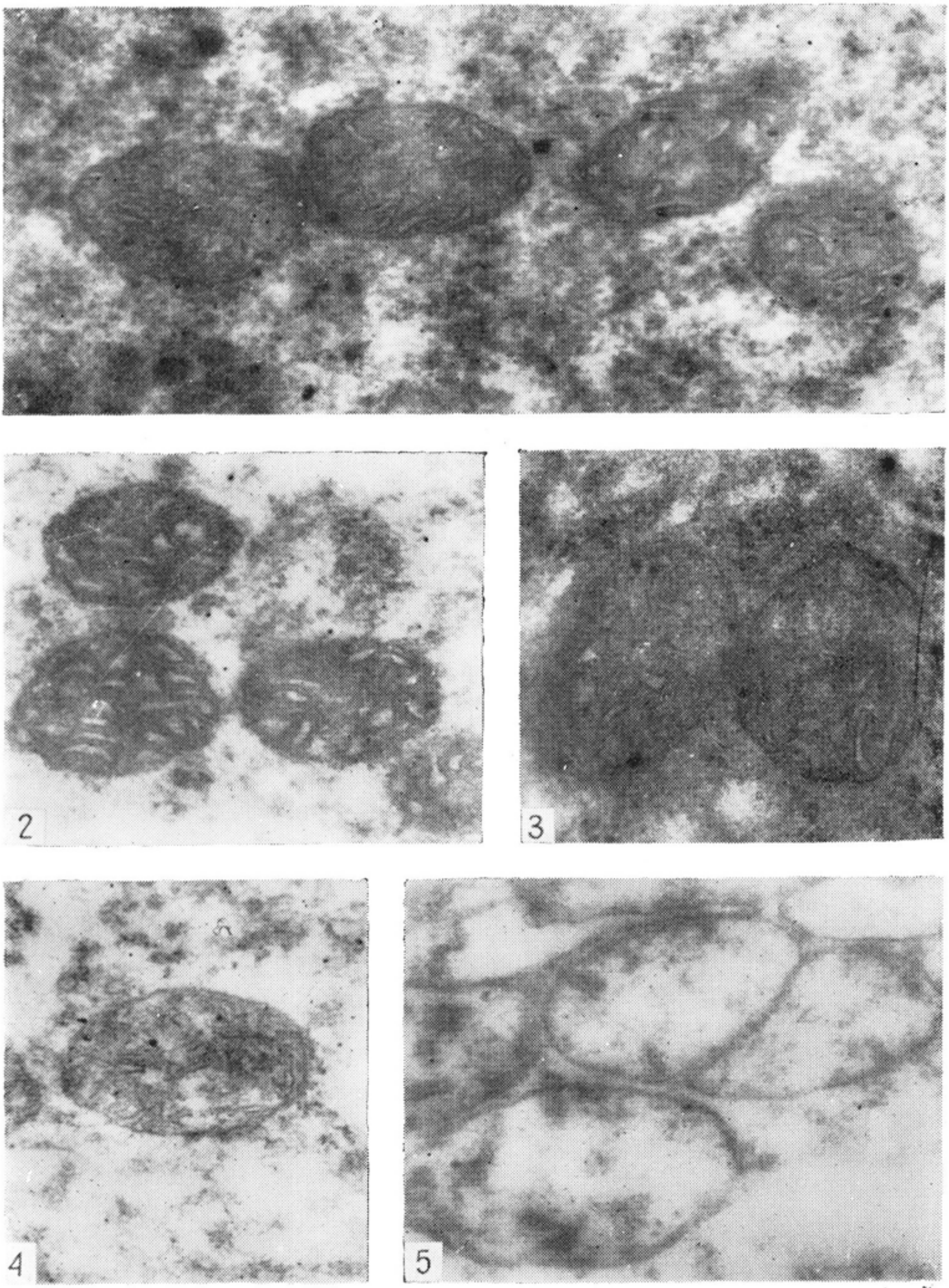
Table 1

Comparison of significance of differences in the number of mitochondria of group IV after 24-hrs. exposure to 0°C

	t value	significance
A. glaucum/TAN 822	1.45	—
A. glaucum/TAN 829	2.38	+
A. glaucum/Lutescens 329	4.81	+
Lutescens/TAN 822	3.20	+
Lutescens/TAN 829	2.36	+
TAN 822/TAN 829	0.908	—

the rate of changes in the shape of the mitochondria exposed to —4°C was also noticeable in the plants previously exposed to 0°C for 7 days (Fig. 3). Similar differences appeared under exposure to 0°C: the elongated mitochondria appeared earlier in *Agropyron* than in wheat. More extensive comparative studies were performed on the rate of changes in the shape of mitochondria of *Agropyron*, Lutescens 329 and of the remaining plants. At first, living material was used and the above described method applied for estimation of the proportions of the four types of mitochondria. The coleoptiles were examined after keeping the seedlings for 20 and 40 mins. and for 3, 4 and 19 hrs. at 0°C. It results from these observations that actually the most efficient reaction to a temperature change occurs in the mitochondria of the most hardy *A. glaucum*, and the slowest change in the unresistant wheat cv. Kooperatorka. The remaining plants could be classified as intermediate between *Agropyron* and Kooperatorka. The classification order of the remaining plants between *Agropyron* and Kooperatorka is identical as regards hardiness and the rate of change. This concerns both the hybrids and the wheats (see Kwiatkowska 1970 b, Fig. 2). Since the differences in the cell picture observed at short time intervals were relatively small, the results obtained on the living material by the above mentioned method could cause doubt, therefore the observations on the rate of change were also repeated on part of the material post vitam. The investigations were made on *Agropyron*, on both the hybrids, TAN 829 and TAN 822, and on their maternal form cv. Lutescens 329 as follows: from the seedlings exposed to 0° for 20 mins., 1 hr. and 24 hrs. preparations were made directly after their removal from the barochamber. They were then inspected in glutaric aldehyde buffered with phosphates to pH 7. In this medium the movement of the cytoplasm ceased immediately, and the picture of the cell remained for about 20 min identical to that during life. The number of mitochondria of the four types distinguished was counted in fields delineated by the graticule micrometer. The fields were chosen at random on the area of all the well preserved cells in the section. For each experimental variant 1000 mitochondria were analysed from 10 plants, by counting 100 of them from each plant. The significance of the differences in the number of mitochondria of group IV (after 24 hrs. at 0°C), most characteristic for this temperature since they only appear in the experimental conditions (cf. Fig. 4 A), was established, and it confirmed the in vivo observations which showed that there

Plate I



Mitochondria ultrastructure of coleoptile epidermis of *Agropyron glaucum* L. fixed in glutaric aldehyde at +1°C with postfixation in osmium tetroxide:

photo 1. in seedlings cultivated under control conditions (25°C in darkness)

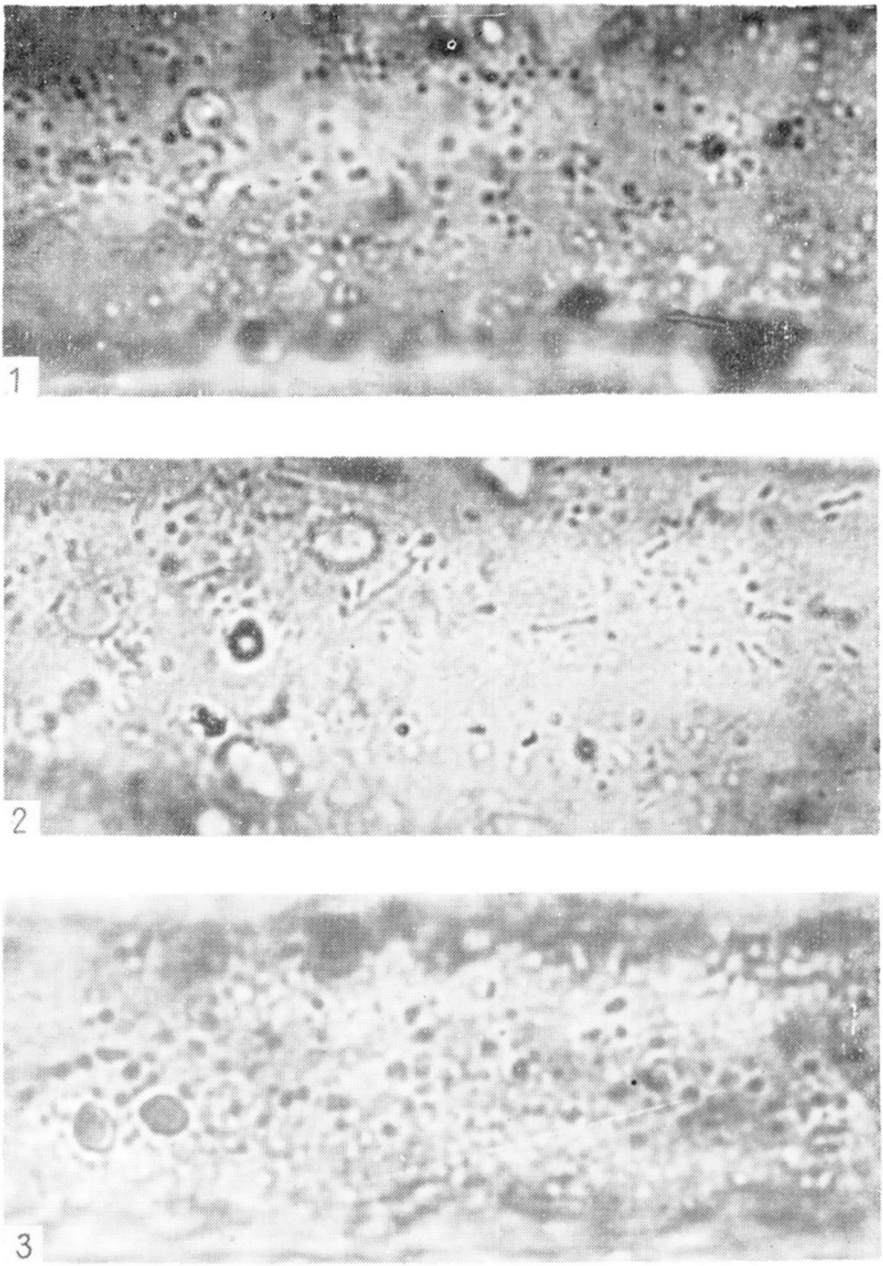
photo 2. subjected to 0°C for 24 hrs.

photo 3. subjected to −4°C for 24 hrs.

photo 4. seedling exposed to 0°C for 7 days

photo 5. after exposure to 0°C for 7 days and to −4°C for another week. The plants exposed to cold were fixed without defrosting. × 45 000

Plate II



Fragments of epidermal cells of *Agropyron glaucum* coleoptiles at a distance of 1.5–2 mm from apex fixed with glutaric aldehyde and stained in the mercury-bromophenol blue reaction:

photo 1. seedling kept in darkness at +25°C
photo 2. after 24 hrs. at 0°C
photo 3. after 24 hrs. exposure to −4°C × 2 500

Table 2

Comparison of significance of differences in dynamics of appearance of group IV mitochondria

	after 20 min		after 1 hr	
	t value	significance	*t* value	significance
A. glaucum/TAN 822	2.88	+	2.90	+
A. glaucum/TAN 829	0.194	−	2.27	+
A. glaucum/Lutescens	6.83	+	2.87	+
Lutescens/TAN 822	2.64	+	0.681	−
Lutescens/TAN 829	4.99	+	4.41	+
TAN 829/TAN 822	2.21	+	5.51	+

is no relation between the number of rod-like mitochondria appearing at 0°C and the degree of hardiness of the plants. It is true that the most significant difference was observed between *A. glaucum*, the most hardy plant, and cv. Lutescens 329 (medium resistant), but TAN 822 less resistant than *Agropyron* is closer to the latter (insignificant difference) than the more hardy TAN 829 (significant difference, Table 1).

The rate of change in the shape of mitochondria in the same plants, just as in vivo observations, showed however, a parallel course with the degree of hardiness to frost: *A. glaucum* and TAN 829 exhibited a stronger dynamics than TAN 822 and Lutescens 329 (Fig. 4 B).

The differences and similarities in the rate of changes were most pronounced when they were expressed as relative values (Fig. 4) assuming the increase or decrease in the number of mitochondria of the different types after 24 hrs. of exposure to 0°C in relation to the control values as 100%.

Then the percentage of these changes 20 and 60 min after transferring the plants to 0°C was calculated (in the calculations the mitochondria of category II were omitted since they are probably transitory forms, and their number at first increased and then decreased reaching, in part of the cases, a value close to the initial one). An analysis of the significance of the differences in the rapidity of appearance of the longest mitochondria, that is of category IV, indicates that similarity is closest between TAN 829 and *A. glaucum*, particularly after 20 mins., and between Lutescens 329 and TAN 822 after one hour of exposure of the plants to 0°C (Table 2). The hybrids differ from one another significantly as regards dynamics.

DISCUSSION

In vivo investigations demonstrated that the reaction to a weak (0°C to —2°C) thermal stimulus or a stronger (—4°C) stimulus of short duration consists in the appearance of elongated mitochondria (Figs. 1 and 2). Longer exposure to —4°C and lower temperatures causes the swelling of the mitochondria.

Observations of the ultrastructure of *Agropyron* mitochondria indicate that the changes in their shape are not parallel to changes in their ultrastructure. The elongated mitochondria forming, as has been mentioned, as the result of the aggregation of spherical mitochondria with the short rods after 24 hrs. of exposure to 0°C, and the mitochondria of the control plants had an identical ultrastructure. It should be added that only a 7-day exposure of the seedlings to 0°C brought about clarification of the mitochondrial matrix. It may thus be supposed that in the first period the aggregation of spherical mitochondria into long rods is above all associated with a reduction of the contact surface with the cytoplasm of the external membrane of the mitochondria, without change in their volume. It would seem that this phenomenon, like the process of active shrinking and swelling of the mitochondria (Lehninger 1964) may be connected with the autoregulation of the biochemical activity of the mitochondria by limiting the permeation of compounds necessary for ATP synthesis from the cytoplasm to the mitochondria, and of the ATP produced in them to the cytoplasm. It is characteristic that the elongation of mitochondria under the action of other factors (e.g. long soaking in water, Buvat 1948; berberine sulphate, Perner and Pfefferkorn 1953; respiration inhibitors and anaerobic conditions, Wrischer and Devidé 1965) described in the literature, occurred simultaneously with the decrease in the intensity of respiration. It also decreases at lower temperatures (James 1953). Another problem is whether the formation of linear mitochondrial aggregates is due to changes in the physical properties of the cytoplasm, or of the mitochondria themselves. Buvat (1953) supposed that the shape of the latter depends on the cytoplasm movement: a slow flow favours the formation of long mitochondria, whereas a rapid movement disrupts the elongated mitochondria into spherical forms and short rods. Actually, a temperature of 0°C which causes the formation of elongated mitochondria, reduces at the same time the intensity of the cytoplasmic flow (Zurzycki 1951; Kamiya, 1959). Nevertheless, the present author observed the formation of very long mitochondria also at the moment of rapid movement of the cytoplasm. This occurred during defrosting of the seedlings which had first been hardened at 0°C for 7 days, and the exposed for 24 hrs. to —4°C (cf. Kwiatkowska 1970 a). Therefore, it is probable that the formation of rod-shaped mitochondria is an active process connected with the intensification in certain conditions of the polar properties of mitochondria. It would seem that this feature is characteristic only of mitochondria, in contrast to swelling which may involve other cell structures in plants chilled below —4°C (Kwiatkowska 1970).

It is known that the swelling of mitochondria is their nonspecific reaction to the influence of various factors. It may be a passive process connected with changes in osmotic pressure, or it may be active — associated with changes in the functional properties of the mitochondria (Lehninger 1964; Wrischer and Devidè 1965; Maszanckij et al. 1965; Cailloux and Genevès 1966; Johnson and Wilson 1967; Earnshaw and Truelove 1968; Yoshida and Sato 1968, Lorimer and Miller 1969 and others). Mitochondria swollen owing to exposure to —4°C in seedlings transferred to this temperature for 24 hrs. directly from a temperatures of +25°C did not exhibit changes in their ultrastructure similarly as those of plants kept for 24 hrs. at 0°C. Only a prolonged action of moderately low temperatures 0°C for 7 days and —4°C for 1 or 7 days produced great transformations in the ultrastructure of mitochondria, manifested in the disappearance of the electron-dense matrix and the reduction of cristae. Mitochondria with a similar appearance isolated from pea seedlings exposed to frost were deprived of the ability of oxidative phosphorylation and characterized by a reduced intensity of respiration (Maszanckij et al. 1965; Ivanova and Semikhatova 1966). The changes described by the above quoted authors were obtained by a short-lasting action of much lower temperatures (—18° to —30° for 15 mins.), or of higher than optimal temperatures. The changes described here in the ultrastructure of mitochondria induced by moderately low temperatures occur almost simultaneously with the process of adaptation of these objects and parallelly to the transformation of the basic cytoplasm which becomes almost electron-dense to electrons. The endoplasmic reticulum also changes undergoing fragmentation and vesiculation (Kwiatkowska 1970 a).

On the basis of up-to-date data it is impossible to decide to what degree these alterations in the ultrastructure of the mitochondria affect their biochemical activity, and whether they are in any way connected with thermal resistance. These problems require further physiological, biochemical and cytological studies. On the other hand, the author believes that the in vivo investigations described here allow to claim that the degree of hardiness of plants depends on the rate at which the changes of shape in their mitochondria occur at low temperature. The mitochondria of plants exhibiting greatest thermal resistance transformed most efficiently (*A. glaucum*, TAN 829). The rate of the transformations decreased parallelly with the hardiness of the cereals.

It would, therefore, seem that the changes observed in the shape of the mitochondria may be considered as active adaptive reactions of the cell to cold, which protect it to some degree from irreversible damage.

SUMMARY

Changes were observed in the shape and size of mitochondria in living cells of the epidermis of the apical part of coleoptiles from seedlings of a number of plants exhibiting various degrees of hardiness, subjected to chilling (0° to −8°C). The following plants were investigated: *Agropyron glaucum*, *Triticum aestivum* var. *lutescens*, cv. Lutescens 329, and their hybrids — TAN 822 and TAN 829 as well as *Triticum aestivum* var. *erythrospermum* cv. Kooperatorka and *Triticum aestivum* var. *velutinum*, cv. Ulyanovka. Detailed observations were performed on the mitochondria of *A. glaucum* and cv. Lutescens 329. In both cases it was found that, irrespective of whether the temperature was lowered suddenly or gradually, the shape of the mitochondria was characteristic for the given temperature.

Two steps in the changes could be distinguished depending on the degree of temperature depression: 1) temperatures within the limits of 0°C to −2°C produced the appearance of elongated mitochondria which were not found in the controls. At the same time the number of mitochondria in the cells diminished, this suggesting that the elongated forms arise by linear aggregation of spherical mitochondria; 2) temperatures of −4°C to −8°C caused the mitochondria to assume spherical forms and swell.

A similar step in the changes of shape occurred also depending on the time of exposure to a temperature of −4°C which, when applied for a short time, caused the mitochondria to elongate

to long rods, and prolonged exposure to cold brought about swelling or a return to the spherical shape.

Changes in the ultrastructure of mitochondria under the influence of the temperatures 0° and −4° appeared only after their prolonged exposure to these temperatures (about 1 week) and did not occur simultaneously with the changes in shape caused by these temperatures. They consist in a clarification of the matrix and reduction of the cristae.

Comparison of the behaviour of mitochondria from plants with a different degree of hardiness showed that they do not change equally under the effect of low temperature. At 0°C a large number of elongated mitochondria is formed in *Agropyron glaucum*, they are less numerous in the hybrids, and their number is smallest in wheats. There are no significant differences in the number of elongated mitochondria between the hybrids differing in hardiness as well as between the three wheat varieties also dissimilar as regards thermal resistance.

The kinds of plants investigated also differed in the rate at which the shapes of the mitochondria changed upon exposure to low temperatures. The strongest dynamics was noted in the most hardy *A. glaucum* and TAN 829, it decreased in the following order: TAN 822, cv. Lutescens 329 and cv. Kooperatorka. The degree of hardiness to frost of the plants decrease in the same order.

The following suggestions are advanced: 1) the hardiness of the plants is related to the rapidity of change in the shape of the mitochondria; 2) the changes observed in the shape of the mitochondria are an active adaptation reaction of the cells exposed to cold.

The experimental part of the work was performed and the electron microscope photographs were taken in the Institute of Cytology and Genetics, Siberian Division, Academy of Sciences of USSR in Novosybirsk. The author wishes to thank the Management of the Institute for making available to her the material and apparatus for performing the investigations.

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Zmiany morfologiczne mitochondriów wywołane działaniem zimna na koleoptyle zbóż o różnej mrozoodporności

Streszczenie

Obserwowano zmiany kształtu i wielkości mitochondriów w żywych komórkach skórki wierzchołkowej części koleoptyli z siewek szeregu roślin o różnym stopniu mrozoodporności, poddanych chłodzeniu (t. 0°C do −8°C). Były to: *Agropyron glaucum*, *Triticum aestivum* var. *lutescens*, cv. Lutescens 329, ich mieszańce — TAN 822 i TAN 829 oraz *Triticum aestivum* var. *erytrospermum* cv. Kooperatorka i *Triticum aestivum* var. *velutinum*, cv. Uljanovka. Szczegółowe obserwacje przeprowadzono na mitochondriach *A. glaucum* i cv. Lutescens 329. Stwierdzono, że u obydwu obiektów, niezależnie od tego, czy temperaturę obniżano skokowo, czy stopniowo, kształt mitochondriów był dla danej temperatury charakterystyczny.

Wyróżniały się dwie fazy zmian, zależnych od stopnia obniżenia temperatury. 1) Temperatury w granicach od 0°C do −2°C powodowały zjawienie się wydłużonych mitochondriów, które w kontroli nie występowały. Równocześnie zmniejszała się liczba mitochondriów w komórce, co sugeruje, że wydłużone mitochondria powstają drogą liniowego łączenia się mitochondriów kulistych. 2) Temperatury od −4°C do −8°C powodowały przyjmowanie postaci kulistej i pęcznienie mitochondriów.

Podobna dwufazowość zmian kształtu mitochondriów występowała również w zależności od czasu działania temperatury −4°C, która przy krótkotrwałym działaniu wywoływała powstawanie mitochondriów w kształcie długich pałeczek, przy dłuższym — ich pęcznienie i ponowny powrót do formy kulistej.

Zmiany ultrastruktury mitochondriów pod wpływem t. 0°C i −4°C pojawiają się dopiero po dłuższym ich działaniu (ok. tygodnia) i nie zachodzą jednocześnie ze zmianami kształtów wywołanymi wpływem tych temperatur. Polegają one na rozjaśnieniu matrix i redukcji cristae.

Porównując zachowanie się mitochondriów roślin o różnym stopniu mrozoodporności stwierdzono, że w warunkach obniżonej temperatury zmieniają się one niejednakowo. Przy t. 0° powstaje u *Agropyron glaucum* duża liczba mitochondriów wydłużonych, nieco mniej u mieszańców, najmniej — u pszenic. Brak jest istotnych różnic w liczebności wydłużonych mitochondriów zarówno pomiędzy mieszańcami o różnym stopniu mrozoodporności, jak wśród trzech różniących się odpornością termiczną pszenic.

Badane obiekty różniły się także szybkością, z jaką zmieniały się kształty ich mitochondriów przy obniżonej temperaturze. Największą dynamikę zmian stwierdzono u najbardziej mrozoodpornego *A. glaucum* i TAN 829, mniejszą u TAN 822, jeszcze mniejszą u cv. Lutescens 329, najmniejszą u cv. Kooperatorka. W tej samej kolejności zmniejsza się stopień mrozoodporności tych roślin.

Wysunięto następujące przypuszczenia: 1) mrozoodporność rośliny pozostaje w związku z szybkością zmian kształtów mitochondriów, 2) obserwowane zmiany kształtu mitochondriów są aktywną, adaptacyjną reakcją komórkową na działanie zimna.

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