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Comparative Studies on the Electrophoretic Patterns of Acid-Soluble Chromosomal Proteins during *Zea mays* Early Stages of Embryo Germination and Root Cell Differentiation

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Abstract. Acid-soluble chromosomal proteins were extracted from purified nuclei, isolated from 3—4 h, 12—14 h and 24—26 h maize embryos, as well as from nuclei isolated from meristematic, elongating and differentiated cells, from 2 and 3-day-old *Zea mays* seedlings primary roots.

Using polyacrylamide gel electrophoresis (urea-acetic acid in the cylindrical gels and slab--SDS-electrophoresis), it was established that germination and root cell differentiation induced changes in some nuclear acid-soluble chromosomal proteins, especially in histone H1.

The results of proteins belonging to the high-mobility group proteins (HMG) and some other acid-soluble proteins with unknown nature for the plants, based on the electrophoretic mobility, were discussed.

The process of seed germination is characterised by activation of RNA and protein synthesis (MAYER and SHAIN 1974), processes associated with the transcriptional activity of chromatin. Undoubtedly, investigations of the changes in the structure and function of the chromatin are necessary in order to clarify how the transcriptionally inactive chromatin in dormant seed embryos becomes active during germination. All of them are connected with the two main classes of chromosomal proteins — histones and nonhistones.

Reports on the changes in chromosomal proteins in the process of seed germination are still scarce and on a limited number of plant species, which determines the necessity of further investigations.

The seed germination after their imbibition is accompanied by de-aggregated chromatin in embryos (SUGITA *et al.* 1980), increased sensitivity of chromatin to the action of DN-ase II (GRELLET *et al.* 1977), decreased ratio of histone H1 to DNA (GRELLET *et al.* 1977, YOSHIDA *et al.* 1979), increased template activity of chromatin (YOSHIDA and SASAKI 1977) and involvement of some nonhistone chromosomal proteins in the inhibition or stimulation of the

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template activity of chromatin (YOSHIDA and SASAKI 1977, SUGITA *et al.* 1979, YOSHIDA *et al.* 1979).

Our purpose was to trace the similarity and diversity in polyacrylamide gel electrophoretic patterns by disc electrophoresis in urea-acetic acid, as well as by slab-SDS-electrophoresis of nuclear acid-soluble chromosomal proteins, extracted from embryos 3—4 h, 12—14 h and 24—26 h after the seeds imbibition, by comparing them with the profiles of the same proteins, isolated from meristematic, elongating and differentiated root cells of 2- and 3-day-old maize seedlings. Such a comparison is grounded on our recent findings concerning the changes which these proteins undergo in the process of root differentiation (MÁRINOVA *et al.* 1981), as well as the possibility of considering these changes from the viewpoint of two types of plant cell differentiation: differentiation at the early germination stages and differentiation in the process of the root cell growth.

MATERIAL AND METHODS

The experiments were carried out with maize seeds (*Zea mays* L. cv. Kneja 2L-611), washed and soaked for 3 h, 12 h and 24 h in tap water, in a thermostat, at a temperature of 25—28 °C. From the imbibed seeds 3—4 h, 12—14 h and 24—26 h embryos were extracted with maximum removal of endosperm and scutelum. Another part of the seeds were left to grow on a moist filter at the same temperature for 48 h and 72 h. Out of the 48—56 h and 72 to 80 h primary root segments containing mainly meristematic (0—2 mm), elongating (2—5 mm) and differentiated (5—15 mm) root cells were cut. The material was fixed in 50% glycerol and stored at —25 °C.

The plant material was homogenized in the grinding buffer according to MULLER *et al.* (1980) with Robot 309 blendor (Poland), twice or three times for 20 s at about 14 000 rev. min^{-1}. All further procedures in the purification of nuclei were performed according to the above-mentioned authors. The purity of the isolated nuclei was checked microscopically and by their DNA, RNA and protein content.

The acid soluble proteins were extracted twice for 30 min with 0.2 M H_2SO_4, after preliminary washing of the nuclei for 30 min with 0.15 M NaCl, 0.01 M Tris/HCl, pH. 7.5. The acid-soluble proteins were precipitated with 4 volumes of absolute ethanol for 48 h at —25 °C and prepared for electrophoresis by the method described by MURRAY and KEY (1978).

All procedures of isolation and purification of the nuclei, the extraction of proteins and their preparation for electrophoresis were performed at 0—4 °C; phenylmethyl-sulfonylfluoride (0.5—1 mM) was added as inhibitor of the proteolytic activity, in the presence of 1% dimethylsulfoxid (v/v) and 10 mM β-mercaptoethanol, both at the stages mentioned, as well as in the electrophoretic fractionation of proteins.

The electrophoretic separation of proteins was done simultaneously following two methods:

1) in cylindrical gels, 120 mm long and 5 mm in diameter, containing 15% acrylamide (m/v) and 2.5 M urea (by PANYIM and CHALKLEY 1969) with 30 to 200 μl proteins per gel. After pre-electrophoresis for 6 h at 40 V, the gels were run with 0.9 M CH_3COOH at 60 V for 22 h or 80 V for 18 h. The gels were stained with Amidoblack 10B for 2 h.

2) by slab-SDS-electrophoresis according to LAEMMLI (1970) with dimensions: 180 mm length, 130 mm height and 1 mm thickness, with a 6% (m/v) acrylamide stacking gel and 18% (m/v) running gel, with 15 to 50 µl protein as a sample. Electrophoresis was run at 40—100 mA (70—160 V) for 4 h and 30 min up to 6 h. The gels were stained with Coomassie brilliant blue R250 (and/or G250) for one night. In both cases the excess dye was removed by diffusion. The stained protein bands were visualized by photographing and recording. Densitometry tracing was performed by scanning at 610 nm by ERI-65 (Zeiss).

RESULTS

From the photoprints, as well as from the densitometric profiles it is seen that the acid-soluble proteins are large in number, even in one-dimensional electrophoresis (Figs. 1 to 4). More than 20 protein bands are seen and can be scanned. Most of the acid-soluble proteins are histones. Alongside with them other basic proteins are extracted.

Histone proteins are identified not only by their solubility and electrophoretic mobility in SDS gels with standard histone proteins of calf thymus, but also by comparison of the electrophoretic patterns with those published in the literature, especially for histones of different maize tissues (TOWILL and NOODEN 1973). These histone proteins are referred to according to the currently accepted terminology as H1, H2A, H2B, H3 and H4. In the electrophoretic profile H3, due to its great quantity, is often recorded together with H2, while H2A and H2B are not separated in either method of fractionation. The ratio of the basic core histones H2A + H2B, H3 and H4 is the same. As regards histone H1, its content in the embryos compared with the remaining histones, is higher than that of the root cells. The total content of H1 tends to decrease during germination (Figs. 1, 2). The decrease of H1 from a meristematic to a differentiated stage of the root cells is expressed even better (Figs. 3, 4). At the same time a variability in the subfractions of H1 can be seen. In the investigated nuclear and acid-soluble chromosomal proteins, isolated from embryos, in both types of electrophoresis three subfractions of H1 (a, b, c) are observed, whose number remains the same in germination, while their ratio varies. The most constant subfraction is H1c, whereas the subfractions H1a and H1b are found in the smallest quantity in 24—26 h embryos. In acid-soluble proteins, isolated from different growth stages of root cells the number of subfractions of H1 is probably 5 (a′, a, b, c, d). In electrophoresis in urea-acetic acid they are three (Fig. 3). Besides the definitely expressed decrease in the quantity of H1 in the cells passing from meristematic to differentiated stage, the ratio of the separate subfractions of H1 is quite varied.

The figures given also show that the histone proteins are accompanied by acid-soluble basic proteins, the proteins concentrated in a greater quantity being marked by arrows. Since so far we have identified them only by their electrophoretic mobility and in accordance with the scanty data of these or similar proteins of plant objects, we find it more appropriate to refer them to one group or another in the discussion.

DISCUSSION

As was shown in the results presented, the main part of acid-soluble proteins are histones. Irrespective of the enormous information on histones, their role in the regulation processes has not been made clear, being still less known of plant objects (SHANNON and DANIEL 1979).

Most constant in the evolution aspect are histones H3 and H4 (GOFSHTEIN 1978). It is well known that the cysteine-containing histone H3 forms dimers on oxydation, which during urea-acetic acid electrophoresis move twice as slowly as the monomeric form. In order to prevent dimerization, in our experiments we added β-mercaptoethanol as a reducing agent, both in the isolation of nuclei and in all analytical procedures. A pre-electrophoresis, before the addition of protein samples, in urea-acetic acid electrophoresis was also carried out, which diminishes the possibility of oxidation of H3 by the persulphate (SHANNON and DANIEL 1979). All this confirms the opinion that H3 is in monomer configuration in our experiments. At SDS-electrophoresis a protein band moves between H3 and H4, which can sooner be referred to H4 as a result of modifying of this histone by acetylation (NADEAU *et al.* 1974). In any case the question of variants of the core histones in plants, as a result of different modification, is not well investigated (RODRIGUEZ and BRANDT 1979). From our experiments very little can be said of histones of the group H2. They do not separate in the methods of fractionation used, and possess an electrophoretic mobility specific for plants H2B and H2A (NADEAU *et al.* 1974, SPIKER 1980). Special attention was devoted in our investigations to histone H1. As is known histone H1 is considered to take part in the condensation of chromatin fibrils, thus enhancing the formation of the high-order organization (RINDT and NOVER 1980). It was already mentioned that histone H1 decreases in germination of pea (GRELLET *et al.* 1977) and wheat plants (YOSHIDA *et al.* 1979), parallel with the de-aggregation of chromatin (SUGITA *et al.* 1980) and the increase of template activity (YOSHIDA and SASAKI 1977). Our results show that in the early stages of germination of maize embryos (up to 26th h) the quantity of H1 in relation to the core histones is relatively high, with a tendency towards decreasing during germination. The number of H1 subfractions in germinating embryos is 3 and does not change until the 24th hour of germination. The changes in the total content of histone H1, compared with the remaining histones, are clearly expressed in the root cells at different stages of growth and differentiation. The quantity of H1 decreases from meristematic to differentiated stage of the root cells. The number of subfractions of H1 in the root cells is probably 5, but their ratio varies.

The fraction which moves at SDS-electrophoresis directly following H1 is of great interest. It tends to increase in the process of differentiation. Similar electrophoretic mobility in animal objects have a number of proteins, among which $H1_0$, which is considered to be related to the process of differentiation, as well as to the protein AK (JENSON *et al.* 1980). SUGITA *et al.* (1979) refer this fraction to H1. Further analysis is necessary for a more definite conclusion about the nature of this fraction.

Attention should be drawn to the protein bands situated before H1 in electrophoresis in urea-acetic acid and after the above-discussed fraction in SDS-electrophoresis (Figs. 1, 2, 3, 4). According to their electrophoretic mobility, they can be referred to the high mobility group proteins (HMG). There

are very fwe data on HMG in plants (SPIKER *et al.* 1978, SUGITA *et al.* 1979). Little is known as yet of the functional role of these proteins, but they have been assumed to have a structural role (RINDT and NOVER 1980). Their presence in the chromatin of germinating maize embryos as well as in large quantities in the root cell chromatin favours the assumption that they are structural proteins.

The comparative analyses of electrophoretic patterns of acid-soluble chromosomal proteins show distinct differences in their total content and especially in the quantity of H1 and in the ratio of its subfractions both in the process of differentiation of the root cells and in the process of germination of maize embryos. The decrease of H1 could be connected with the processes of decondensation of chromatin and its activation in the two model systems of plant cell differentiation in connection with the physiological processes taking place in them. Further studies are needed to elucidate the functional role of acid-soluble histone and nonhistone proteins, characterized by us in this study according to their electrophoretic mobility.

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Figures at the end of the issue.

BOOK REVIEW

MONTEITH, J., WEBB, C. (ed.): SOIL WATER AND NITROGEN IN MEDITERRANEAN-TYPE ENVIRONMENTS. Developments in Plant and Soil Science Volume 1. Martinus Nijhoff / Dr. W. Junk Publishers, The Hague—Boston—London 1981. 338 pp., 125 Dfl.

This book is compiled from the selected reviews presented at a workshop, organized by the "International Center for Agricultural Research in the Dry Areas" (ICARDA) with the help of the "United Nations Development Program" (UNDP) at Aleppo (Syria) in January 1980. For increasing the quantity and availability of food in the Mediterranean region, ICARDA is developing a special project on "Increasing the Fixation of Soil Nitrogen and the Efficiency of Soil Water Use in Rainfed Agricultural Systems in the Countries of North Africa and Western Asia", as soil water and nitrogen are considered tc be among the main limiting factors to production in this area. In planning and for realization this project, ICARDA has called on the leading scientists from a number of countries to give the benefit of their experience and advice on methods and priorities on which this specific research can be based and conducted. The workshop was organized for the presentation and exchange of information, discussions and recommendations for project guiding. The book contains 11 reviews, each written by a specialist whose remit it was to review his particular area of interest. The first three reviews are devoted to analysis of the agro-climatology of the zone studied (A. H. Kassam), environmental resources and restraints (R.C.G. Smith and H.C. Harris), and to the present farming systems and possibilities of their improving (D. Gibbon). The next two reviews deal with water dynamics in the soil-plant-atmosphere system (J. T. Ritchie) and with plant-water relations and adaptation to stress (N. C. Turner and J. E. Begg). The subsequent two articles describe nitrogen accession, cycles of transformation and its loss in soils of the arid region (P. L. G. Vlek, I. R. P. Fillery and J. R. Burford), and efficiency of nitrogen use in crop systems (R. Novoa and R. S. Loomis). The last group of four chapters covers modelling the interaction of water and nitrogen (H. van Keulen), optimizing the use of water and nitrogen through soil and crop management (F. E. Bolton),optimizing the use of water and nitrogen through breeding of crops (R. A. Fischer) and possibilities for plant improvement for semi-arid rangelands (D. A. Johnson, M. D. Rumbaugh and K. H. Asay). The concluding summarization "Epilogue: themes and variations" (J. L. Monteith) is followed by "Recommendations" which arose from the workshop papers and discussions. A sole index contains subject items, author's and plant names.

The book provides a record of the current state of knowledge of soil, climate, soil water and soil nitrogen availability, fundamental not only for the mentioned regions but also for areas of similar type occurring practically on every continent. It reviews all principles by which soil, water and nitrogen relations can be studied in every environment and thus it provides a very useful general reference book on these subjects.

JARMILA SOLÁROVÁ (*Praha*)

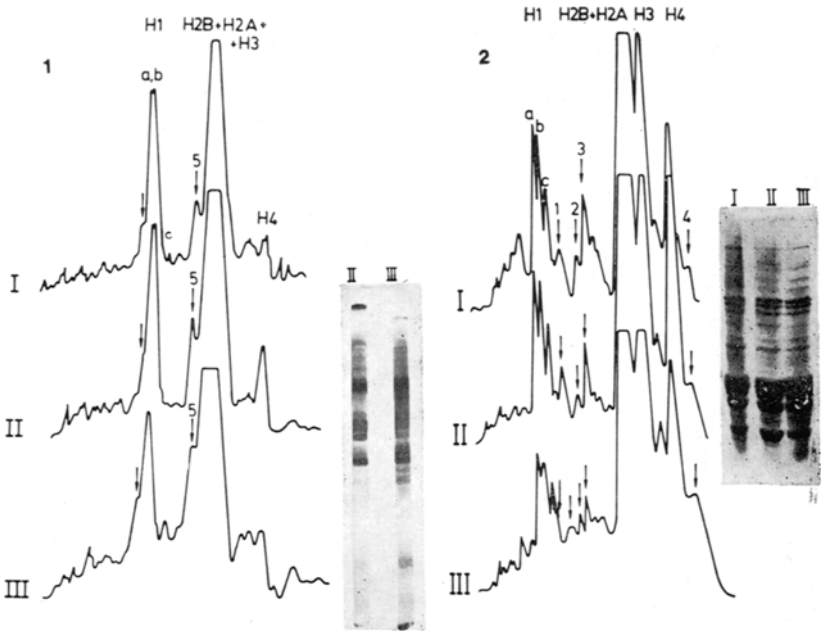

Fig. 1. Urea-acetic acid polyacrylamide gel electrophoretic patterns of nuclear acid-soluble chromosomal proteins, extracted from 3—4 h (I), 12—14 h (II) and 24—26 h (III) maize embryos.
Fig. 2. SDS-polyacrylamide gel electrophoretic patterns of acid-soluble chromosomal proteins, extracted from 3—4 h (I), 12—14 h (II) and 24—26 h (III) maize embryos.

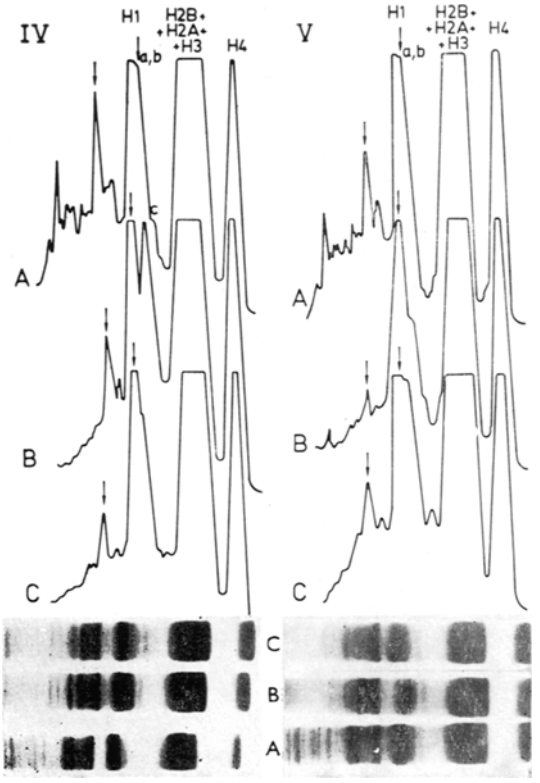

Fig. 3. Urea-acetic acid polyacrylamide gel electrophoretic patterns of nuclear acid-soluble chromosomal proteins, extracted from meristematic (A), elongating (B) and differentiated (C) root cells, from two (IV) and three (V) days old maize seedlings.

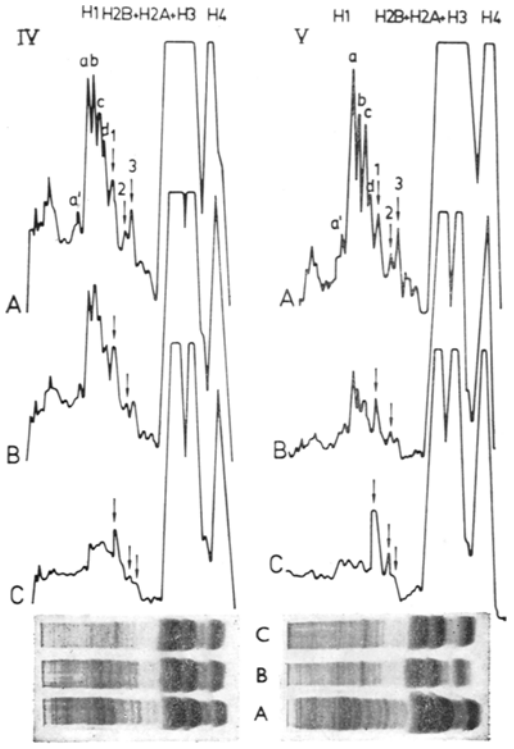

Fig. 4. SDS-polyacrylamide gel electrophoretic patterns of nuclear acid-soluble chromosomal proteins, extracted from meristematic (A), elongating (B) and differentiated (C) root cells, from two (IV) and three (V) days old maize seedlings.