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Features of the formation of *Arg-X* proteolytic system of cellular nuclei during germination of wheat seeds

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Abstract

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The processes of assembly and disassembly of chromatin with the participation of the nuclear proteome are the most important part of the regulatory network that controls the movement of cells the on cell cycle. In this aspect, histones play an important role, since their synthesis is coordinated with DNA synthesis and any changes in their sequence or structure can lead to an effect on cellular metabolism. The aim of the work was to study the dynamics of the activity of *Arg-X* proteolysis in nonhistone and histone fractions of nuclear structures (nucleoplasm, chromatin, nuclear matrix) during germination of wheat seeds under conditions of deacetylation inhibition of nuclear proteins (sodium butyrate). Seeds were germinated in distilled water (control) and in 0.004 mM NaB (sodium butyrate). Cell nuclei were isolated from germs, cleared, and then nucleoplasm, chromatin, nuclear matrix were extracted by increasing ionic strength of solution. From isolated supramolecular structures, non-histone proteins were separated from histones by using ion exchange chromatography. The *Arg-X* proteolytic activity was assessed by cleavage of *Arg-X* bonds in the arginine-enriched protein protamine. The duration of mitosis phases under conditions of deacetylation inhibition of nuclear proteins was determined. It is shown that *Arg-X* proteolysis appears in the fractions of nonhistone proteins and core histones (H2A + H2B; H3 + H4), mainly in the H3 + H4 block at the level of chromatin tightly bound. In addition, the increasing in *Arg-X* proteolytic activity (24 h-48 h) coincides with the beginning of proliferative activity, which may be due to chromatin rearrangements in cells during germination of seeds. The presented results can be considered as one of the illustrations of a complex regulatory proteinase network formed in cell nuclei, which can participate in proteolytic processing of histones, thereby affecting chromatin reorganization

Keywords: Arg-X proteolysis; cell nuclei; histones; sodium butyrate; *Triticum aestivum* L.

Introduction

It is known the nucleus is the regulatory center of the eukaryotic cell, and nuclear proteins are a vital component of cell nuclei. However, while the plant genome is well understood at the DNA level, information on plant nuclear proteins remains extremely insufficient (Petrovská et al., 2015). Nuclear proteins make up approximately 10-20% of the total number of cellular proteins and form regulatory networks functioning in this organelle (Narula et al., 2013). Nuclear proteins are poorly researched due to their low content and complexity of isolation, and often the information obtained using highly reliable methods does not illustrate the functional purpose of nuclear proteins. Consequently, it is necessary to investigate the compositional divergence of the nuclear proteome on individual phases of the cell cycle, or obtained from various types of tissues and stages of differentiation of cells and tissues (Petrovská et al., 2015).

It is known that the processes of assembly and disassembly of chromatin with the participation of the nuclear proteome are the most important part of the regulatory network that controls the movement of cells the on cell cycle. The assembly of nucleosomes is a complex and strictly regulated process in eukaryotes, which is crucial for maintaining genomic integrity. In this aspect, histones play an important role, since their synthesis is coordinated with DNA synthesis and any changes in their sequence or structure can lead to an effect on cellular metabolism (Duronio & Marzluff, 2017). It has been proven that nucleosome control plays an important role in regulating the expression (Merkulova et al., 2013). It has been shown that almost all the histones are proteolytically clipped and histone proteases, clipping off the histone tails, create a new nucleosome (Azad & Tomar, 2014), thereby affecting the chromatin dynamics (Azad et al., 2018). In addition, the histone substrate has the ability to independently regulate and modulate the activity of proteases acting on it (Azad & Tomar, 2014).

Of all the modifications, acetylation is known to have the highest potential to cause chromatin unwinding. Neutralization of the positive charge of lysine in the tails of histones affects the destabilization of internucleosomal contacts and, consequently, the structure of chromatin (Rosa & Shaw, 2013). It is known that histone deacetylation is an important feature of the chromatin assembly process, as the removal of acetyl group restores the positive charge of lysine residues and the electrostatic attraction between DNA and histones (Annunziato & Hansenf, 2000; Koprinarova et al., 2016).

The susceptibility of histones to proteolysis in chromatin can be regulated by adding or removing acetyl or methyl groups on lysine residues near the cleavage sites. Modifications of some lysine residues can either make lysine residues unrecognizable for proteases, thereby preventing splitting at this site, or they can alter the interaction of histones with DNA or proteins (Mandal et al., 2014). Post-translational modifications of histones are the main characteristics of epigenetic regulatory networks. Regulatory proteolysis of histones is one of these modifications, which remains insufficiently studied (Vossaert et al., 2014; Sun-Ju & Kyunghwan, 2018).

The aim of the work was to study the dynamics of the activity of *Arg*-X proteolysis in nonhistone and histone fractions of nuclear structures (nucleoplasm, chromatin, nuclear matrix) during germination of wheat seeds under conditions of deacetylation inhibition of nuclear proteins (sodium butyrate).

Materials and Methods

Highest-quality seeds of wheat (*Triticum aestivum* L.) cv. Artemovka (spring) were selected for this investigation. Seeds were kindly provided by the collection of the "All-Russian Research Institute of Plant Industry". The wheat seeds were germinated at 22 ± 1 °C in the dark. Only morphologically identical seedlings were used for this experiment. For soaking the seeds and the germination in the control variant was used distilled water and in the test was used 0.004 mM sodium butyrate (Ivanova & Vafina, 2009a). The embryo was divided from the endosperm 24 hours after the start of the experiment. Cell nuclei were isolated from entire seedlings using the method described by Ivanova and Vafina (1991). Nuclear structures were isolated from purified cell nuclei by increasing the ionic strength of solutions. The nucleoplasm (Np) fraction was extracted by 0.14 M NaCl. The fraction of chromatin loosely bound (Chromatin I) was extracted by 0.35 M NaCl. The fraction of chromatin tightly bound (Chromatin II) was extracted by 2 M NaCl. Fractions (Chromatin I and Chromatin II) differ by the ratio active and inactive genes; localization in the nucleus; sensitivity to action of dissociates substances, nucleases; ability to undergo conformation transitions (Karavanov & Afanasjev, 1983; Mironov et al., 1987; Ivanova & Vafina, 1992). The nuclear matrix (NM) was extracted using 6 M guanidine hydrochloride (GuHCl) with 0.004% -mercaptoethanol (Ivanova & Vafina, 1992). The amount of protein in the nuclei and nuclear fractions was determined using the Bradford method (1976) with some modifications (Ivanova & Vafina, 1992). Histone and non-histone protein fractions were gotten by chromatographic fractionation of nuclear structures (nucleoplasm, chromatin I, chromatin II, nuclear matrix) on columns with Amberlite IRC-50 (Serva, Heidelberg). Amberlite IRC-50 was prepared using the method from Ivanova & Vafina (2009b). Proteins were eluted in a step-wise gradient (6.0, 8.9, 10.6, 13, and 40%) of GuHCl. The amino acid composition of the fractions obtained after elution of the basic chromatin proteins with increasing concentrations of guanidine hydrochloride proves that the obtained proteins correspond to histones HI, H2A and H2B, H3 and H4 (Ivanova & Vafina, 2009b).

Activity of *Arg*-X proteolysis was evaluated by cleavage of *Arg-X* bonds in the arginine-enriched protein protamine salmine A1 ("Merck", Germany), a molecule that consists of 33 amino acids (22 molecules of Arg, 4 Ser, 3 Pro, 2 Glu and 1 Val) in all fractions of the nuclei (Ivanova & Vafina, 1992). *Arg-X* proteolytic activity was calculated in nanomoles of arginine per second per microgram of protein (nmol Arg/s/μg protein). The determination of the duration of mitosis phases was carried out on the root meristem of wheat seedlings. The slides were made by the squash technique using the meristem of the main root (Kalashnik, 2008).

The experiments were repeated three times with at least three chemical repeats and two independent biological sam-

ples analyzed at each time. Data are presented as arithmetic means +/- the standard deviation (SD).

Results and Discussion

As it is known, the start of growth processes is carried out by stretching the cells. In dry mature seeds, the germ cells may be in the G1 and G2 states, however, cells in the G1 state, presumably the least vulnerable state of the meristematic cell, predominate (Danovich et al., 1982). In germinating wheat germ, five peaks of mitosis were recorded (Yadav & Das, 1974), first in the root, then in the leaflets, and only after the root reached 1.5 cm in the stem meristem (see Danovich et al., 1982). This root length corresponds to seedlings approximately 42–48 h. The transition from a "dry" resting embryo to a metabolically active seedling followed by the cell divisions is accompanied by large-scale chromatin reorganization. Post-translational modifications of histones play a crucial role in regulating the structure and function of chromatin. The histone octamer has a unique structure, the complexity of which is enhanced by the N-terminal tails of histones. It has been proven that electrostatic interactions of the phosphodiester DNA chain with arginine residues in the histone octamer are most important for the organization of DNA in nucleosomes. Only 14 arginine residues are needed to maintain DNA wrapping in nucleosomes (Wolffe, 1998). Histones are rich in lysine and arginine. Many chromatin modifiers target these amino acid residues. Acetylation is one of the most studied PTM, which occurs on all histones. Acetylation occurs on specific lysines, which are located in the N-terminal domains of the core histones. Potentially, the nucleosome has 28 or more acetylation sites. It has been shown that the acetylation of N-terminal domains of the core histones interferes of the interaction with proteins and/or DNA and thereby destabilizes the organization of higher-order chromatin, thereby promoting of transcription (Davie, 1997; 2003). It is known, that the N-terminal tail of histone H3 (1-23) contains 5 lysine and 3 arginine residues, which will be positively charged at physiological pH. The H4 sequence, 1-20, also contains 5 lysine and 3 arginine residues and histidine. Thus, the hyperacetylation of key nucleosomal histones H3 and H4 can significantly reduce the electrostatic interaction between the histone core and DNA (Anderson, 1980) and activate transcription (Mandal et al., 2014). The dynamically acetylated isoforms of H3 and H4 histones correlating with transcriptional activity have a higher rate of degradation (Cox & Day, 1981) and are not detected during mitosis (Kruhlak et al., 2001). It is known that histone deacetylation is an important mechanism involved in chromatin assembly. Chromatin in the presence of deacetylase inhibitors is sensitive to DNase 1, does not form higher order structures (Annunziato, 2015; Le Beyec, 2007), and stabilization of condensed mitotic chromosomes decreases (Koprinarova, 2016; Patzlaff et al., 2010).

A classic technique for studying the functional activity of deacetylases is the using of their inhibitors, for example, sodium butyrate. Today, the best known effect of sodium butyrate is its ability to induce acetylation of histones (Kumar et al., 2007) and activate the chromatin transcription, with histones H3, H4 and H2B experiencing the greatest effect (Haan et al., 1986). In addition, sodium butyrate inhibits cell proliferation by blocking the cell cycle at the G1/S and G2/M boundaries at low concentrations (Kumar et al., 2007). Mainly post-translational modifications occur in the N-terminal tails of histones. It was previously shown that N-terminal tails of histones H3 and H4, which are removed by trypsin-like proteolysis, have a high degree of conservatism and are acetylated (Bohm et al., 1981). It has been proven that clipping the histone H3 and H4 tails affects the structure and dynamics of chromatin and the efficiency of transcription (Azad et al., 2018). The susceptibility of histones to proteolysis in chromatin can be regulated by adding or removing acetyl or methyl groups on lysine residues near cleavage sites and post-translational modifications can regulate the rate of proteolysis (Mandal et al., 2014). It is known that polycationic compounds cause spiralization and block chromatin despiralization. For example, arginine added to isolated nuclei in the interphase leads to the formation of prophase-like filaments (Mazia, 1961).

In the course of this work, it was shown (Figures 1 and 2) that *Arg-X* proteolysis activity has its own characteristics of localization in the superstructures of cell nuclei during the growth and development of wheat germ. Figures 1 and 2 present the dynamics of the *Arg-X* protease activity in nonhistone and histone fractions at the level of the superstructures: nucleoplasm, loosely and tightly bound chromatin and nuclear matrix in the control variant (Figure 1) and under conditions of deacetylation inhibition of nuclear proteins (Figure 2).

It is shown that *Arg-X* proteolysis appears in the fractions of nonhistone proteins and core histones (H2A + H2B; H3 + H4), mainly in the H3 + H4 block at the level of chromatin tightly bound (Chromatin II) (Fig. 1). Histones H3 and H4 play a key role in the structure and dynamics of chromatin due to their location in the octamer. The tails of histones are also involved in nucleosome–nucleosome interaction for establishing transcriptionally repressive chromatin, referred to as heterochromatin (Yu et al., 2011; Mandal et al., 2014). It is possible that active proteolytic processes localized in the (H3 + H4) block are associated with the processes of chromatin

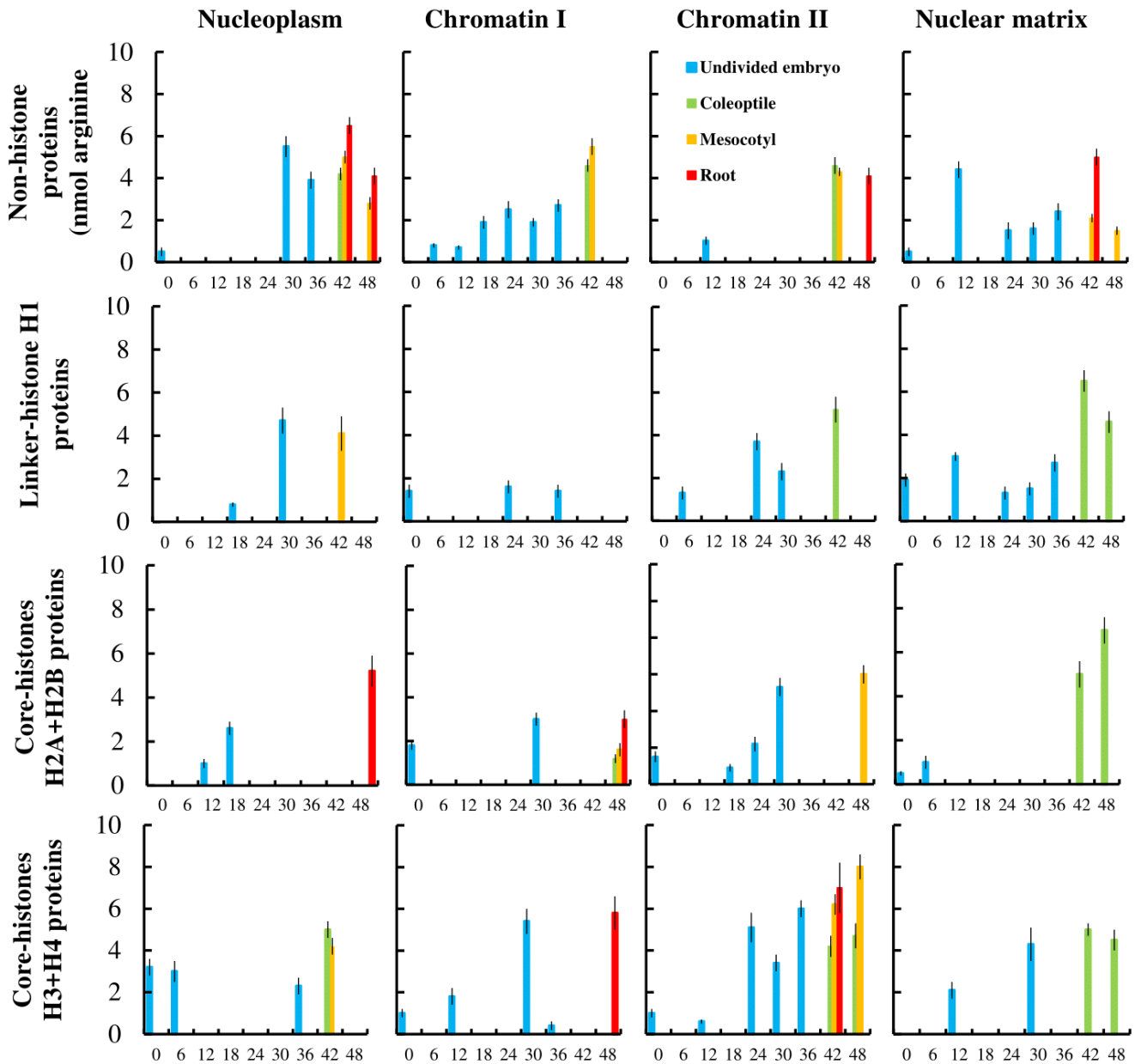

Fig. 1. The activity of *Arg*-X proteolysis in non-histone proteins (NHP), linker histone (HI) and core histones (H2A+H2B, H3+H4) of superstructures of cell nuclei: nucleoplasm, chromatin loosely bound (chromatin I), chromatin tightly bound (chromatin II), and nuclear matrix of mature seedlings in normal conditions of germination.

reorganization in proliferating cells (24 hours – 48 hours). It is known from the literature that acetylation of H3 and H4 histones is associated with the transcriptional activity of chromatin (Anderson, 1980) and that their value rapidly reduces during mitosis (Kruhlak et al., 2001). Under the conditions of inhibition of the deacetylation of nuclear proteins (Figure 2), it was shown that *Arg-X* proteolytic activity was not detected in many fractions compared with the control. It is known that the rate of histone turnover in proliferating cells is higher compared to non-proliferating cells, which is

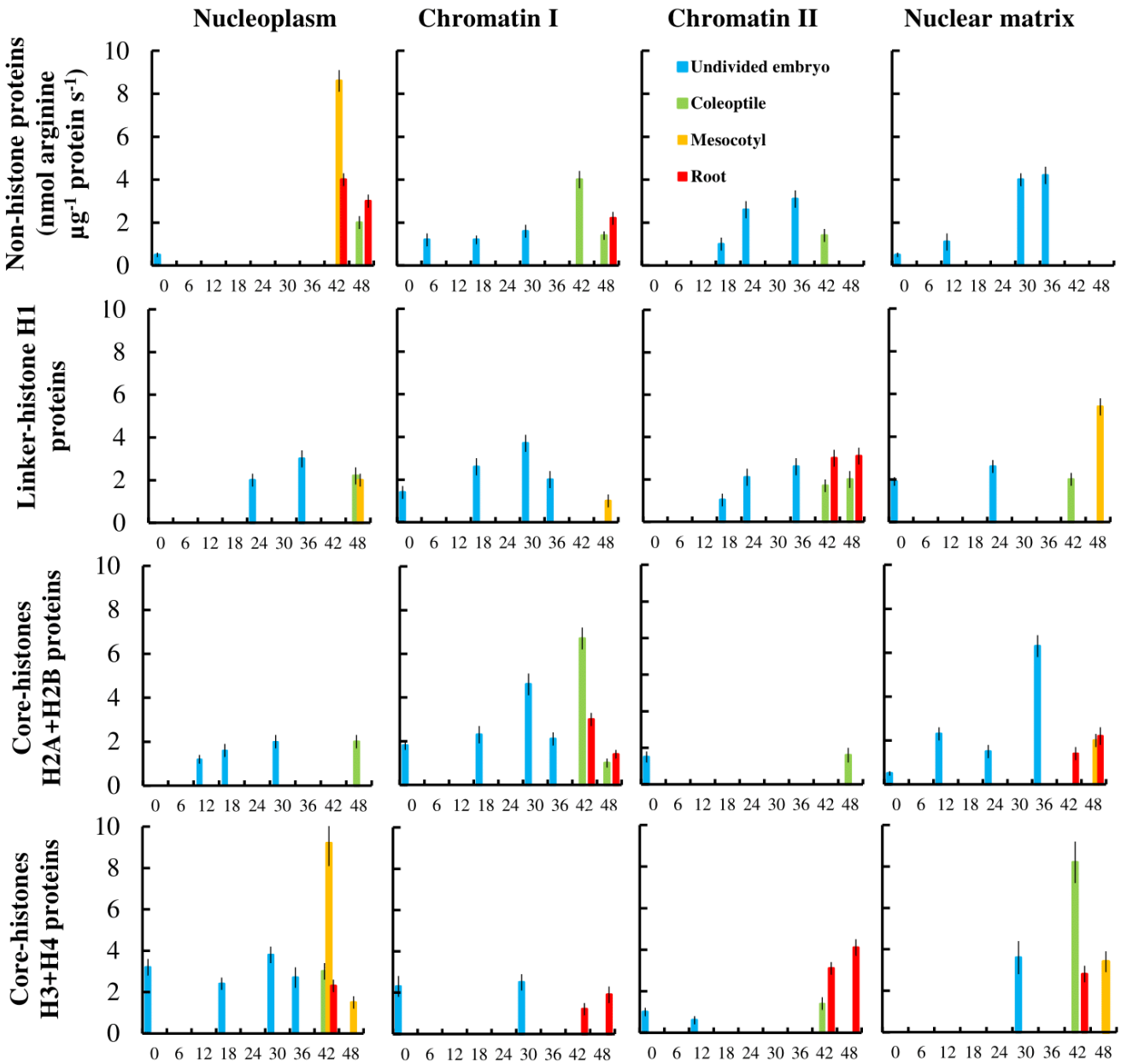

Fig. 2. The activity of *Arg*-X proteolysis in non-histone proteins (NHP), linker histone (HI) and core histones (H2A+H2B, H3+H4) of superstructures of cell nuclei: nucleoplasm, chromatin loosely bound (chromatin I), chromatin tightly bound (chromatin II), and nuclear matrix of mature seedlings in normal conditions of germination in conditions of deacetylation inhibition of nuclear proteins (sodium butyrate)

accompanied by the presence of higher proteolytic activity in histones associated with chromatin, which indicates the possible role of histone proteases in histone metabolism (Purohit et al., 2012). It is possible that the decrease in the rate of histone turnover as a result of the influence of sodium butyrate on the mitotic activity in cells shows a decrease in the activity of proteolytic enzymes (Vafina et al., 2017).

Activity of *Arg-X* proteolysis is manifested in fractions of histones and non-histones at the level of the nuclear matrix (Figures 1 and 2). We assume that *Arg-X* proteolysis activity

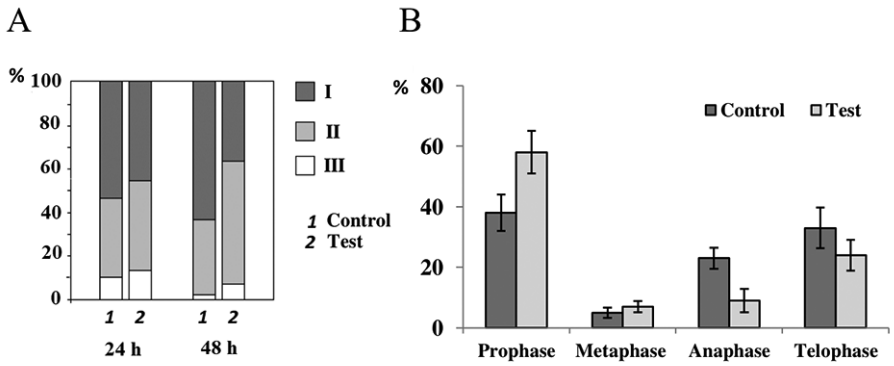

Fig. 3. Analysis of the population of seedlings (A) and the duration of mitosis phases on root meristem of 2-day-old seedlings of wheat (B).

Distilled water was used as the control (1) and 0.004 mM sodium butyrate as the test (2).

24 h: I- Normally growing seedlings – total length is 0.3 – 0.5 cm (taken in the experiment), II- lagging seedlings – the total length up to 0.3 cm, III- non germinated embryos.

48 h: I- Normally growing seedlings – the length of the main root is 1,5-2 cm, the length of the coleoptiles is 0.8-1 cm (taken in the experiment); II- lagging seedlings – the length of the main root up to 1,4 cm, the length of the coleoptile up to 0.7 cm, III – non-germinated embryos.

at the level of the nuclear matrix may be associated with cell layers that actively grow by stretching, as cells retain their ability to stretch in the presence of sodium butyrate (Chiatante et al., 1986).

Furthermore, analysis of Figures 1 and 2 shows that a high level of *Arg-X* proteolytic activity coincides with the beginning of proliferative activity in cells. The chromatin containing the clipped histones becomes structurally rigid and can persist for a long time until the clipped histones are degraded and replaced with intact histones. Some studies show that clipped histones are present in the promoters of the genes required for the differentiation process (Azad et al., 2018).

The morphophysiological data of the population of seedlings grown in water (control) and in the presence of sodium butyrate (test) present on the Figure 3A.

The percentage of lagging seedlings (test) increases 48 hours from the start of soaking. The data on the duration of mitosis phases in the apical meristem of the main embryonic root (Figure 3 B) demonstrate that cells accumulate at the prophase stage in the presence of sodium butyrate. It is known that deacetylation of chromatin proteins is an essential process for stabilizing mitotic chromosomes (Koprinarova et al., 2016) and that deacetylation of histones H4 and H3 is important for progression through mitosis (Li et al., 2005). Apparently, nuclear proteins retain their acetylation status as a result of exposure to sodium butyrate, which complicates the process of chromatin compaction in the early stages of mitosis (Zhiteneva et al., 2017).

Conclusion

The presented results can be considered as the illustrations of a complex regulatory proteinase network (Turk et al., 2012; Narula et al., 2013), formed in cell nuclei that can participate in proteolytic clipping of histones, thereby affecting chromatin reorganization.

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