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Effect of Nickel on Growth, Proliferation, and Differentiation of Root Cells in *Triticum aestivum* Seedlings

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Abstract—The effect of 10^{-6} and 10^{-4} M $NiSO_4$ on cell growth, proliferation, and differentiation was studied over 48 h in seminal and lateral roots of five-day-old *Triticum aestivum* seedlings. 10^{-6} M $NiSO_4$ did not significantly affect the root system, whereas 10^{-4} M $NiSO_4$ inhibited its development. However, 10^{-6} M $NiSO_4$ disturbed the contacts between the groups of closely related cells of the rhizodermis in the meristem. In the exodermis, an additional layer of cells was formed. At the nickel concentration of 10^{-4} M, cell divisions in the outer layers of the root cells and metaxylem ceased earlier than in other root tissues positioned both centripetally and acropetally. Differentiation of protophloem sieve elements was completed in the meristem but at a greater distance from the root tip. Cell elongation started at the same distance from the root tip as in control plants. The rate of elongation decreased, and acropetally it stopped. Therefore, the cells of the xylem and metaphloem started to differentiate, and primordia of lateral roots were initiated and formed closer to the root tip. At a lethal concentration (10^{-4} M), nickel induced necroses of elongating cells of the endodermis and pericycle. Nickel is supposed to enter the tissues of the central cylinder predominantly via the protoxylem and rapidly translocate along the xylem. As a result, the incubation of the roots at this concentration for 48 h almost did not affect the development of the phloem and probably sugar unloading, that makes possible to maintain the growth of meristematic cells and the cell division of the most important tissues for longer time.

Key words: Triticum aestivum - root - meristem - primordium - nickel - growth - cell proliferation - cell differentiation

INTRODUCTION

Numerous investigations concern the effect of heavy metals on plant development. Several monographs and reviews have been published for the last 15 years [1–6]. Heavy metals were found to rapidly affect the morphogenesis of the plant root system by suppressing growth of the main root and lateral roots. To a lesser extent, metals affect the development of lateral root primordia. Many researchers believe that heavy metals inhibit root growth by suppressing division and elongation of the cells and reducing the cell length that completed growth. However, there are only sparse data as to which of these processes is most sensitive to heavy metals. Even relatively low concentrations of heavy metals in the medium induce a decrease in the density and a collapse of root hairs [2]. The excess of heavy metals reduces the value of the mitotic index [5, 7], increases duration of the mitotic cycle [8], induces chromosomal aberrations, and disturbs the formation of the fragmoplast [9]. However, the values of the mitotic indices and the capacity of the proliferating pool in the meristem may decrease not only due to prolongation of some phases of the mitotic cycle but also as a result of consecutive cessation of mitoses (and/or of the entire cycle) of the cells in different tissues as heavy metals enter the root. This possibility was not investigated because, as a rule, the preparations of squashed segments of root tips or fragments of the meristem were analyzed. Upon the effect of zinc and cadmium on maize seedling roots, cell divisions in the cortical part of the quiescent center became more active [10].

In the literature, there are scarce data concerning the effect of heavy metals on the development of cells in various root tissues. It was found that, in the seedlings of zinc-sensitive cultivar of *Festuca rubra* L., zinc excess in the medium reduced the length of the meristem and shifted the zone of root-hair appearance and differentiating xylem cells to the root tip [11]. However, the absence of detailed investigations concerning the effect of heavy metals on the development of conducting tissues in the root makes it difficult not only to understand the causes of a decrease in the rate of its growth and/or its recovery after the removal of the metals from the medium but also to determine the pathways of translocation of metals along the root. The metal-induced inhibition of initiation of lateral root primordia is also poorly investigated. It is only known that the treatment of *Pisum sativum* L. roots with 10^{-5} M $CdCl_2$ resulted in an increase in the levels of DNA, RNA, protein, and polyamines in differentiating cells, as well as

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transition of bound gibberellins to free state and an increase in the number of lateral roots [12].

Among heavy metals, nickel is notable for especially high toxicity, inhibition of plant growth [13], and the high rate of its transport to the aboveground organs [14–17]. Nickel induces various disturbances in the structure and functioning of root cells (vacuolation of meristematic cells, plasmolysis of elongating cells, and changes in the structure of nucleus and nucleolus) [18]. Depending on nickel concentration in medium, two main mechanisms of its effect on the development of the seedling root system are possible [18]. One of them is inhibition of cell divisions, and the other one is inhibition of cell elongation. Inhibition of root growth in the seedlings of *Zea mays* induced by nickel may be accounted for by a decrease in the mitotic activity of the cells [19, 20]. Investigations of the effect of nickel on cell elongation produced contradictory results. For instance, Robertson [19] found that the cells of the elongation zone became rigid and lost their ability to grow. L'Huiller *et al.* [20] concluded that the elongation of cells was not inhibited. At the lethal concentration of $NiSO_4$ (2×10^{-5} M) in the medium, growth of *Zea mays* seedling roots was inhibited as early as 8 h after the beginning of the exposure to metal, although slow growth of the root still continued at its higher concentrations [19]. However, the rapid suppression of root growth may be largely caused by the inhibition of cell elongation [21]. Therefore, it is important to understand the relationship between cell growth in the meristem and elongation zones in response to the excess of nickel in the medium. In all the papers referred above, the effect of nickel on the nature of cell growth along the root longitudinal axis, proliferation and differentiation of the cells of various tissues was not investigated.

In this work, we studied the cell growth in the meristem and elongation zone, as well as proliferation and differentiation of the cells in seminal and lateral roots of *T. aestivum* seedlings at two $NiSO_4$ concentrations (10^{-6} and 10^{-4} M) in the medium, which considerably differed in the effect on the development of the root system.

MATERIALS AND METHODS

Investigations were conducted with the roots of wheat (*Triticum aestivum* L.) seedlings, cv. Leningradka. The caryopses were treated with nystatin for 30 min, stratified at 4°C for 15 h, and germinated in the petri dishes in darkness at 26°C. After 30 h, the seedlings were transferred to the growth vessels. Seedling roots were plunged into an aerated 1/4 Knop solution. Subsequent growth occurred at the same temperature, a 16-h photoperiod, and an irradiance of 23 W/m^2. Five days after the start of germination, some seedlings with the average root length of 70 mm were transferred to 1.4-l vessels with the same medium (control plants) or with the medium containing $NiSO_4$ at the concentrations of 10^{-6} or 10^{-4} M (experimental plants). Each vessel accommodated 50 seedlings. Two days after the start of the experiment, we measured the length of central seminal root of every seedling. Then these roots were fixed according to Navashin (1% chromic acid, 40% formaldehyde, and glacial acetic acid, 10 : 4 : 1) and stained with the Schiff reagent [22]. From each central and lateral root, the meristematic zones and the segments located above (4 and 3 mm in length, respectively) as well as the segments carrying primordia of lateral roots were excised. Root segments were stained with alcian blue 8 GS. The permanent 7-μm-thick preparations of longitudinal and cross sections of root segments were mounted. In order to investigate the effect of studied concentrations of nickel on the cell growth along the longitudinal axis of the root tip, changes in the length of cells in the central file of the metaxylem were analyzed in 25 control and 14 experimental plants as well as in 10 cell files of outer layer of the cortex (located under the exodermis) in 5 control and 5 experimental plants. The cell length was measured using an eyepiece micrometer with an accuracy of 1 μm. The table shows the means and their standard errors.

RESULTS

Effect of nickel on morphology of the root system. At the concentration of 10^{-6} M, $NiSO_4$ did not considerably affect the development of the seedling root system (Figs. 1a, 1b). In control and experimental plants, the length of roots was essentially the same and during two days increased by 30 mm on the average. There were no distinct differences in the number and length of lateral roots. At the concentration of 10^{-4} M, $NiSO_4$ inhibited not only growth of the seminal root (whose length within the same period increased by only 6 mm) but also the growth of lateral roots (Fig. 1c).

Effect of 10^{-6} M $NiSO_4$ in the medium on the structure of seminal and lateral roots. The lengths of division and elongation zones in seminal and lateral roots of experimental and control plants did not differ. The distance from the root tip, at which the initiation of lateral root primordia occurred, and the rate of their development did not change either. However, in the meristem of both seminal and lateral roots, nickel disturbed contacts between the groups (pairs, triplets, tetrads) of closely related cells in the files of the rhizodermis and caused death and hypertrophy of individual cells (Figs. 2a, 2c). This disturbance did not result in cessation of cell divisions in the rhizodermis but activated them in the exodermis (and other layers of the cortex) and altered the pattern of cell divisions. As a result, additional layers of the cells were formed (Figs. 2a, 2c).

Effect of 10^{-4} M $NiSO_4$ in the medium on the cell division in the meristem of the seminal root. The analysis of distribution of mitoses within the tissues showed that the cells of the rhizodermis, exodermis, and middle layers of the cortex (except for distal cells of the files of these tissues), as well as peripheral cells of calyptrogen ceased to divide upon the treatment with nickel. The cells of the endodermis, pericycle, stelar parenchyma,

Effect of nickel on cell growth along the longitudinal axis of root tips in 7-day-old *Triticum aestivum* seedlings

Distance from the initial cell of the file, μm	Cell length in the central file of the metaxylem, μm		Cell length in the outer cortical layer, μm	
	without treatment	10^{-4} M $NiSO_4$	without treatment	10^{-4} M $NiSO_4$
0 (initial cell of the file)	18.4 ± 0.8	24.1 ± 1.6	19.5 ± 1.2	20.3 ± 1.7
0–99	12.7 ± 0.3	18.0 ± 0.6	14.9 ± 0.5	22.2 ± 0.8
100–199	11.9 ± 0.3	17.4 ± 0.5	14.1 ± 0.5	20.4 ± 0.8
200–299	16.3 ± 0.4	19.7 ± 0.6	14.1 ± 0.5	20.1 ± 0.8
300–499	26.8 ± 0.6	31.0 ± 1.1	14.1 ± 0.3	22.9 ± 0.7
500–699	47.9 ± 1.6	55.0 ± 2.5	14.3 ± 0.3	23.7 ± 1.0
700–899	99.1 ± 4.6	101.6 ± 5.0	24.0 ± 0.9	29.9 ± 1.2
900–1299	237.3 ± 13.4	168.3 ± 7.4	45.2 ± 1.8	43.1 ± 1.9

Note: The table shows the means and standard errors of the cell length in the central file of the metaxylem in 25 control and 14 experimental plants as well as in 10 files of outer cortical layer (located under the exodermis) in 5 control and 5 experimental plants. The experimental plants were grown in the medium containing $NiSO_4$ during 48 h.

and protophloem companion cells continued to divide, although the frequency of divisions and the length of the division zone in these tissues decreased (Figs. 2c, 2d). In the division zone of these tissues, there were no cells whose length was much greater than the length of dividing cells. The cells of the quiescent center and distal cells of the central cylinder tissue files, as well as initial cells of the columella preserved the ability to divide. In the central file of the metaxylem, there were no mitoses. A proximal dividing cell located in the pericycle at a distance of 890 μm from the boundary between the root cap and the root body. After the cessation of cell divisions in the rhizodermis and in the layers of cortex, the cells became vacuolated and vacuolation spread centripetally and acropetally. In three roots out of 14 examined, the process of vacuolation spread up to the initial cells of the cortex files. Mitoses were absent from the meristem of these roots.

Effect of 10^{-4} M $NiSO_4$ in the medium on the pattern of cell growth along the longitudinal axis of the seminal root. Changes in the length of cells of the central file of the metaxylem along the longitudinal axis of the root tip are shown in Fig. 3 and in the table. In the roots of experimental plants, the length of initial cells of the files and the cells located at a distance of 0–199 μm from them was 1.3–1.5 times greater than in the roots of control plants (table). In the roots of experimental plants, at this distance the cells of other tissues continued not only dividing (see above) but also growing. Therefore, an increase in the average length of the metaxylem cells in this region of the roots of experimental plants could be caused by cessation of cell divisions, whereas their growth still continued. In the next regions of the metaxylem file, at a distance of 200–1299 μm from the initial cell, the length of cells gradually increased in the roots of both control and experimental

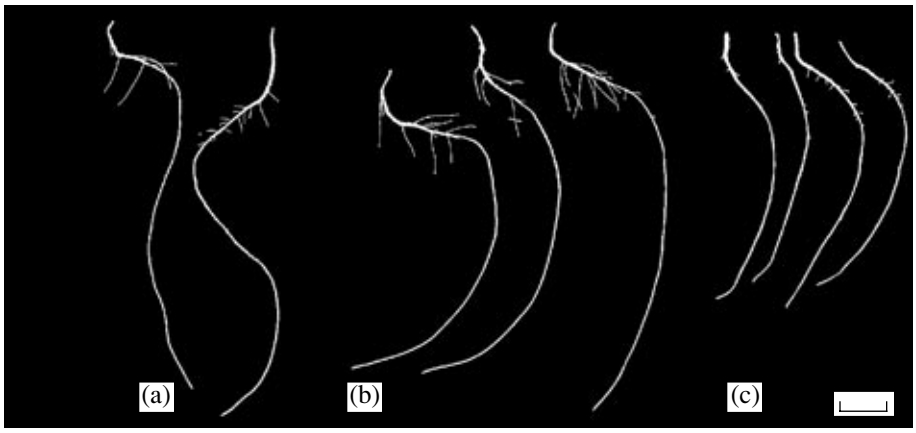

Fig. 1. The roots of seven-day-old *T. aestivum* seedlings grown on the media with various concentrations of $NiSO_4$ during 2 days. (a) No treatment; (b) 10^{-6} M $NiSO_4$; (c) 10^{-4} M $NiSO_4$. The scale bar corresponds to 10 mm.

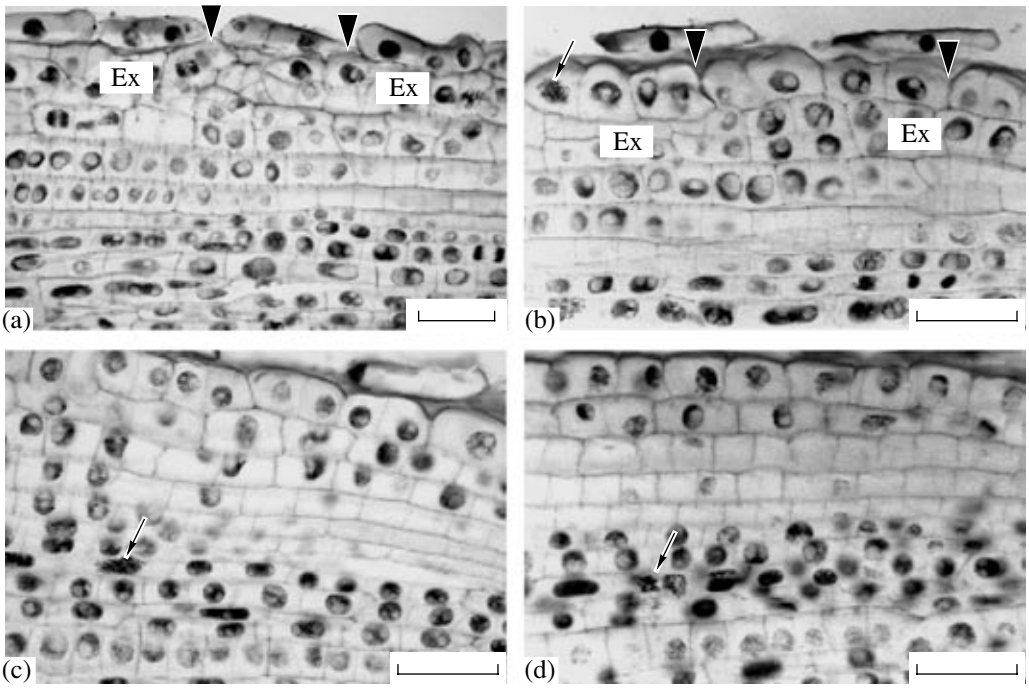

Fig. 2. Longitudinal sections of the meristemic areas in the roots of seven-day-old *T. aestivum* seedlings grown on the media with various concentrations of $NiSO_4$ during 2 days.

(a, b) 10^{-6} M; (c, d) 10^{-4} M. (a, b) disturbance of intercellular contacts in the rhizodermis (triangle) and formation of additional cell layer in the exodermis; (b) hypertrophied cell of the rhizodermis in prophase (arrow); (c) division of the pericycle cell (arrow); (d) division of the protophloem companion cell (arrow). Ex—exodermis. The scale bar corresponds to 50 μm.

plants. In control plants, an increase in the length of metaxylem cells in these regions is related to the cessation of cell divisions and endoreduplication cycles [23]. However, the difference in the cell lengths between the roots of experimental and control plants gradually reduced. At a distance of 900–1299 μm from the initial cell, the length of cells in the roots of experimental plants was much shorter than in control plants. Such a pattern of changes in the cell length in the roots of experimental plants may depend on a decrease in the relative rate of growth with an increase in the distance from the root tip caused by the inhibition of root growth or be accounted for by prolongation of the mitotic cycle and termination of cell divisions in the metaxylem in acropetal direction. In order to resolve this dilemma, we investigated changes in the cell length of outer cortical layer at a distance of 0–1299 μm from the initial cells in the roots of control and experimental plants (table). In the latter, the cell division was inhibited essentially along the entire root meristem. Our data showed that the length of initial cells in the cortex files in the roots of control and experimental plants was the same. This means that the initial cells of the cortex files in the roots of experimental plants continued to proliferate. In the roots of control plants, at a distance of 0–99 μm from the initial cell, the length of cells considerably decreased and remained almost unchanged at a distance of 100–699 μm from the initial cell. In the roots of experimental plants at a distance of 0–699 μm from the initial cell, the cell length essentially did not differ from the length of the initial cell and 1.4–1.7 times exceeded the length of cells in the roots of control plants. The ratio between the lengths of cortical cells in the apical half of this region of the roots of experimental and control plants was the same as in the metaxylem cells at a distance of 0–199 μm where the cells of this tissue in the roots of control plants divided. However, in the basal half of this region, the proportion between the lengths of cortical cells increased. Apparently, this is accounted for by the fact that not only in radial but also in acropetal direction the divisions of cortical cells were inhibited. Thus, nickel did not affect the relative rate of cell growth along the meristem. However, it could be much lower than in the roots of control plants. The presence of nickel in the medium also affected the growth of meristematic cells in the radial direction, which led to a decrease in the diameter of the root. For instance, its diameter at the end of the meristem in control and experimental plants was 362 ± 5 and 336 ± 6 μm, respectively. In the roots of control and experimental plants at a distance of 700–899 μm from the initial cells, the elongation of the cells started. This means that the presence of nickel in the medium did not reduce the length of the meristem. In the roots of experimental and control plants, at a distance of 900–1299 μm from the initial cells, the ratio between the lengths of cortical

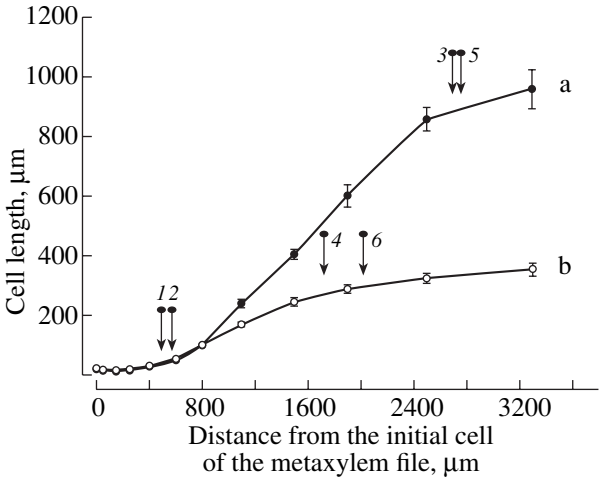

Fig. 3. Changes in the cell length in the metaxylem central file and distances at which differentiation occurs in the root tips of control plants and in seven-day-old *T. aestivum* seedlings grown during 2 days on the medium with 10^{-4} M $NiSO_4$.

Black dots (a)—no treatment; white dots (b)—10^{-4} M $NiSO_4$. The arrows show: (*1, 2*) degradation of the nucleus in the protophloem sieve elements in control and experimental plants, respectively; (*3, 4*) beginning of formation of secondary thickenings in the protoxylem cell walls in control and experimental plants, respectively; (*5*) beginning of root hair growth in control plants; (*6*) resumption of cell divisions in stelar parenchyma and pericycle in the roots of experimental plants.

cells was 0.95; for metaxylem cells, the ratio was 0.69. In the regions located above, the difference between the lengths of metaxylem cells in the roots of experimental and control plants increased (Fig. 3). This may be accounted for by the fact that the relative rate of elongation in the experimental plants was slower than in the control plants. In the region from 2900 to 3700 μm, the length of cells in the roots of experimental plants was 2.7 times shorter than in the roots of control plants.

Effect of 10^{-4} M $NiSO_4$ in the medium on cell differentiation. In the roots of control plants, the beginning of secondary cell-wall thickening in the protoxylem and the emergence of root hairs pointing to the cessation of cell elongation occurred at a distance of 2680 ± 30 and 2750 ± 40 μm, respectively, from the boundary between the root cap and root body (Fig. 3). In the roots of experimental plants, cell elongation ceased much closer to the root tip than in control plants. In the protoxylem of experimental plants, the formation of secondary thickenings of cell walls started at a distance of 1680 ± 50 μm, whereas root hairs in the apical segment of the root did not emerge (Fig. 3). Differentiation of the protophloem sieve elements in the roots of experimental plants was completed at a greater distance from the root tip than in control plants. The first anucleate cells of the sieve elements were located at a distance of 587 ± 11 and 494 ± 8 μm, respectively, from the boundary between the root cap and root body (Fig. 3). In ten roots of experimental plants out of 14 examined, at a distance of 2.2–3.8 mm from the boundary between the root cap and root body, the structure of the endodermis cell walls changed in such a way that they were stained with the Schiff reagent. In these cells, pycnosis of the nucleus occurred. In the root region located above (4–7 mm), necroses of the cells occurred first in the endodermis and the inner layer of the cortex, and then in the pericycle (Fig. 4a). Moreover, the growth of root hairs started. Differentiation of protoxylem cells was completed, and the formation of secondary thickenings in the peripheral metaxylem and degradation of the nuclei in the metaphloem already started. In the roots of experimental plants, at a distance of 7–10 mm from the root tip, the changes in the structure of the cell walls in the endodermis and pericycle were not observed, and pycnotic nuclei were absent. The cells of the central metaxylem had thicker cell walls than in the root segments located below. At this distance, lateral root primordia began emerging on the surface of the seminal root (Fig. 4b).

Effect of 10^{-4} M $NiSO_4$ in the medium on initiation and development of lateral roots. In the roots of experimental plants, primordia of lateral roots of the first order located much closer to the root tip than in the roots of control plants. In some roots, the first cell divisions in stelar parenchyma and pericycle, as well as primordia at the earliest stages of development, occurred already at a distance of 2.2–2.3 mm from the boundary between the root cap and root body (Fig. 3). These dividing cells located closer to the root tip than pycnotic nuclei of endodermal cells occurred. At a distance of 4–7 mm from the root tip, primordia of lateral roots emerged. However, primordia at the earliest stages of development were absent. The cells of the endodermis, which did not participate in the development of primordia, had a disturbed structure of the cell wall and pycnotic nuclei. Some cells of the pericycle were undergoing mitosis or completed the first division. At the same time, the cells of stelar parenchyma divided actively. In primordia of lateral roots and in the lateral roots, which just emerged on the surface of seminal roots of experimental plants, cell division was not inhibited (Fig. 4b). Some cells of the cortex located nearby the primordia were undergoing mitosis (Fig. 4c).

In the lateral roots of the first order with the length of more than 2 mm, distribution of mitoses in the meristematic tissues was the same as in the seminal root. In the lateral roots of the first order with the length of about 2 mm, at a distance of 1.2–1.4 mm from the boundary between the root cap and root body, the initiation of lateral root primordia of the second order started. In longer lateral roots, at a distance of about 2 mm from the boundary between the root cap and root body, lateral root primordia of the second order occurred (Fig. 4d). In the root region where these primordia were located, the cells of all the tissues were much shorter than those located below and above. However, the size of the exodermal cells located above

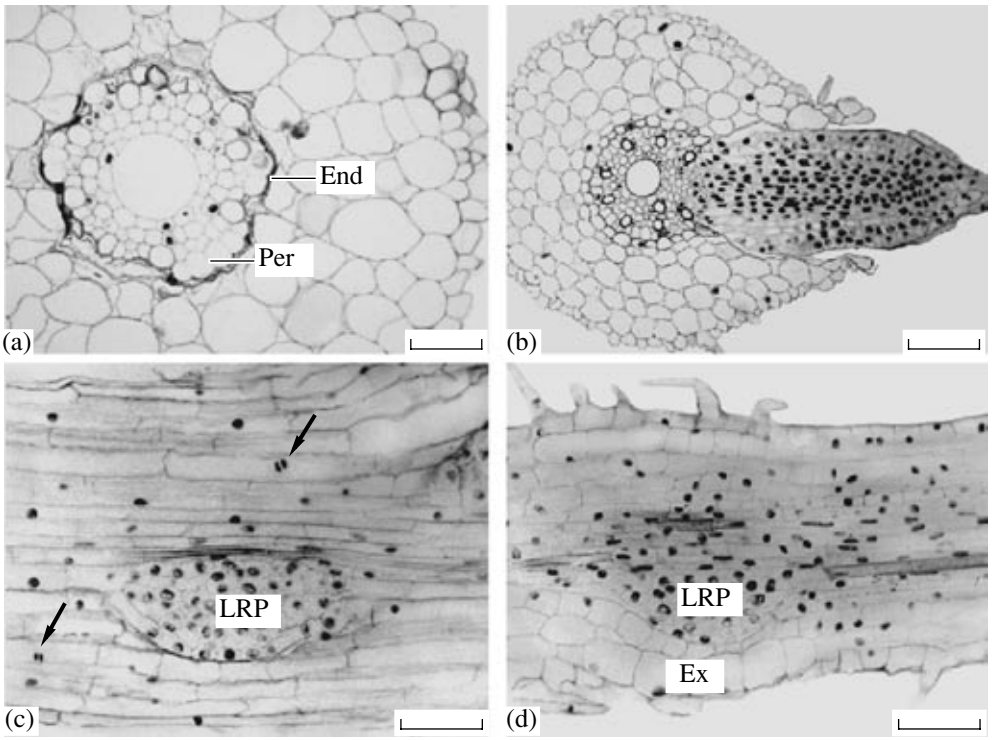

Fig. 4. Sections of the areas of the roots of seven-day-old *T. aestivum* seedlings grown during the latest 2 days on the medium containing 10^{-4} M $NiSO_4$.

(a, b) Cross sections of the root at a distance of 6 and 10 mm from its tip, respectively; (b) primordium of the lateral root has just emerged on the surface of parent root; (c) longitudinal section of the root at a distance of 7 mm from its tip (arrows show the dividing cortical cells located nearby the primordium of the lateral root); (d) lateral root at a distance of 2 mm from its tip with a primordium of the lateral root of the second order. End—endodermis; Ex—exodermis; Per—pericycle; LRP—lateral root primordium. In panel (a), the scale bar corresponds to 50 μm; in panels (b–d), to 100 μm.

the primordium considerably increased in the radial direction, which caused the formation of a swelling in this region of the root. This region carried numerous hypertrophied root hairs.

DISCUSSION

The results of this work confirm the assumption [18] that there are two major mechanisms of the effect of nickel on the development of the seedling root system. Nickel high (lethal) concentrations inhibit both cell division and elongation. According to our results, the main reason for a sharp retardation of root growth at high (10^{-4} M) nickel concentration in the medium is a decrease in the relative rate of cell elongation and its cessation in the acropetal direction. The inhibition of cell division and elongation appeared to start as early as during the first day of incubation of the roots in the presence of nickel. Cessation of divisions in the rhizodermis, cortex (except endodermis), and even in the central file of metaxylem neither stopped the growth of cells in the meristem, nor changed the distance from the root tip where the cells proceeded to elongation. We believe that cessation of divisions in the other tissues as well was not the reason for the cessation of the root growth. In the roots of *Zea mays* seedlings grown in the medium with the lethal nickel concentrations up to 5×10^{-3} M), mitoses in the meristem were not observed [19]. In this case, the increment in the root length amounting to 10% of the increment in the root length of control seedlings could be accounted for not only by elongation of the cells occurring for some time but also by the growth of cells in the meristem. The rate of meristematic cell growth in the radial direction decreased. The reasons for the cessation of cell growth in the meristem are not known so far. Probably, the growth stopped as a result of numerous disturbances of the cell structure and metabolism induced by nickel ions, like it was assumed in respect of other heavy metals that affect growth but do not exert a specific effect on cell division [24].

The results of investigation of the effect of nickel on cell elongation at the concentrations inducing inhibition of root growth by 50% within 2–3-day-long growing of *Zea mays* seedlings, turned out to be contradictory [19, 20]. However, inhibition of the lateral root growth and an increase in their density recorded by L'Huillier *et al.* [20] point to a deceleration of cell elongation. The absence of differences in the length of root cells that completed growth in control and experimental

plants of *Z. mays* grown in the medium containing nickel [25] may be due to a high concentration of Ca^{2+} ions in the medium [19]. At the concentration of 16–32 mg/l, Ca^{2+} ions almost completely protected root growth from the suppressing effect of nickel [19]. However, the mechanism of this phenomenon is not clear so far. Korner *et al.* [26] did not observe a competitive inhibition of nickel uptake induced by Ca^{2+} ions in the excised roots of *Hordeum vulgare* L. These researchers assumed that Ni^{2+} ions are translocated by the same mechanism as Cu^{2+}, Zn^{2+}, and Co^{2+} ions.

Our results show that the inhibition of cell divisions in the root tissues of *T. aestivum* induced by 10^{-4} M nickel occurred both centripetally and acropetally. In the course of incubation of roots with nickel, in the rhizodermis and the layers of cortex (except initial cells of the files and endodermis), mitotic cycles stopped, and the cells became vacuolated. In other root tissues (except xylem), the length of the zone of divisions decreased, and dividing cells occurred seldom. A sharp decrease in the number of dividing cells in the meristem may be only accounted for by a considerable prolongation of the interphase of the mitotic cycle. This is suggested by the fact that the cells longer than dividing cells, i.e., those that stopped passing through the cycle, were absent. An increase in the duration of mitotic cycle mainly at the expense of the phase G_1 induced by Zn^{2+} ions was found in the root meristem of sensitive and tolerant cultivars of *Festuca rubra* L. [8]. The causes of preservation of the ability to divide in the cells of endodermis, pericycle, and other tissues of the central cylinder, as well as for acropetal cessation of cell divisions in the meristem, are not known so far. They may be caused by specific features of nickel uptake and its translocation along the root. However, some other reasons may also exist since 10^{-4} M $NiSO_4$ inhibits growth of the root system, and upon a prolonged incubation this concentration turns out to be lethal.

Dynamics of nickel influx to the root tip is essentially not investigated. According to Seregin *et al.* [25], after two days of incubation of the roots of *Zea mays* seedlings at various concentrations of $Ni(NO_3)_2$ (15, 20, 25, and 35 μM), nickel was detected in all root tissues. In 7 days, its level in tissues considerably increased. In the root tip (5–7 mm in length), the accumulation of nickel was most pronounced in the inner layers of cortex, as well as in endodermis and stelar parenchyma.

It is not known which root zone is most active in the nickel uptake. This work suggests that even the low nickel concentration was toxic for growing cells of the rhizodermis not protected by the root cap. Even at the concentration of 10^{-6} M, nickel disturbed the contacts between the groups of closely related rhizodermal cells in the meristem, bringing about hypertrophy and even death of individual cells (Figs. 2a, 2b), which facilitated penetration of the metal via the apoplast. However, it is not clear so far whether the activation of cell divisions in outer layers of the cortex and the process of regeneration are accounted for by the disturbance of intercellular communications in the rhizodermis or the relations between them are quite opposite. Probably, nickel was predominantly absorbed by the cells of the meristem and elongation zone (Figs. 2a, 2b). However, acropetal cessation of cell divisions in tissues observed upon the action of a high nickel concentration (10^{-4} M) suggests that it was most actively absorbed by the elongation zone. From this zone, nickel could be translocated to the meristem. This was accompanied by the increase in its concentration, and acropetal cell divisions were inhibited. In the wheat roots, round the periphery, the pericycle is interrupted by the protoxylem that has contacts with the endodermis. Therefore, nickel moved from the endodermis (where Casparian strips are not yet formed) to the tissues of the central cylinder predominantly via the protoxylem and rapidly appeared in the central file of the metaxylem, suppressing cell divisions. This is indicated by the fact that, in the zones of cell divisions of the cortex and metaxylem, the ratio between cell lengths in the roots of experimental and control plants was essentially the same (see table). Since nickel ions arrived to the central cylinder predominantly via the protoxylem, their accumulation in the pericycle was considerably lower than in the cells of the inner cortex and endodermis. Therefore, cell necroses in the pericycle occurred much later (and located higher along the root) than in the inner cortex and endodermis and started first in the cells that have contacts with the protoxylem (Fig. 4a). The translocation of nickel ions to the central cylinder did not inhibit the development of the protophloem, although the differentiation of sieve elements was completed at a greater distance from the root tip than in control roots (Fig. 3). Probably, unloading of sugars was not inhibited either, what may account for a prolonged growth and cell divisions in the meristem.

The results of this work show that nickel induced termination of cell elongation in the acropetal direction accompanied by an acropetal dislocation of the place where differentiation of xylem and metaphloem cells started. The zone of cell division resumption in the stelar parenchyma, pericycle, and endodermis related to initiation of lateral root primordia also displaced acropetally. In the root regions where the cells of the endodermis experience necrosis, some cells of the pericycle and stelar parenchyma divided but the development of primordia was not initiated. This indicates that nickel ions do not exert influence on the mechanisms triggering the resumption of the mitotic cycle in relation to initiation of lateral root primordia. In the root zones where the initiation of primordia started earlier than the cells of the endodermis became affected by necrosis, nickel did not inhibit their subsequent development in the seminal root. Probably, this is accounted for not only by the fact that primordia are surrounded with an envelope consisting of the walls of disintegrated cortical cells,

but also by the absence therein of differentiating xylem cells, along which nickel could enter the primordia.

The cells of mature tissues are much less sensitive to the presence of nickel in the medium [19]. According to our data, the structure of the cells that completed elongation before the incubation with nickel did not change (Fig. 4b), and the cortical cells located near the growing primordia of lateral roots were able to resume divisions (Fig. 4c). Weak staining of the cells in the rhizodermis and cortex (except inner layers) pointing to an insignificant content of nickel was observed in the root tip of *Z. mays* seedlings even 7 days after the beginning of incubation with nickel [25].

Thus, the results of this work show that like the ions of other heavy metals [5, 10, 24] the nickel ions exert a series of nonspecific effects on the structure of plant root system. They induce vacuolation of cortical cells and their elongation; reduce the length of cell division zone in the endodermis and central cylinder (although the distance from the root tip where the cells begin elongation remains the same); reduce the number of layers of cortical cells; displace to the root tip the zone where differentiation of xylem and metaphloem cells starts as well as initiation and development of primordia of lateral roots occur. Specific influence of nickel ions on the development of wheat root system depends on their toxic effect on growing cells as well as on their penetration to the tissues of central cylinder predominantly via protoxylem and subsequent translocation along the xylem. As a result of such a peculiarity of nickel ion translocation, the development of phloem in the meristem and probably the unloading of sugars are somehow protected from their influence, which makes it possible to maintain the growth of cells in the meristem and divisions of the cells of the most important tissues for longer time.

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