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Original Research

Changes of Physiological and Genetic Indices of *Lycopersicon Esculentum* Mill. by Cadmium under Different Acidicity and Nutrition

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Abstract

Changes in physiological and genetic indices of *Lycopersicon esculentum* Mill. due to the impact of cadmium at different substrate acidity and nutrition were studied under controlled conditions in phytotron. The amount of photosynthetic pigments, stem diameter, sap flow rate, the mitotic index of cells and inhibition of cell mitosis were investigated. Cadmium in acidic environment produced a very toxic effect on growth, the synthesis of chlorophylls and carotenoids and stem diameter, sap flow rate of *L. esculentum*. Cadmium suppressed the mitotic index of cells and disorganized normal mitosis. The mitosis with anomalies (chromosome breaks, fragmentation, bridges, chromosome eliminations and abnormal nucleus divisions) was observed in meristem cells of roots of *L. esculentum*. It was concluded that nutrient deficiency led to evident plant growth retardation, and higher nutrient favoured plant growth under the effect of cadmium.

Keywords: *Lycopersicon esculentum*, cadmium, substrate acidity, nutrition, plant resistance

Introduction

Plant growth and development is greatly affected by increased environmental pollution [1-3] and investigations of integrated impact of natural and anthropogenic factors on vegetation have become of particular importance. It was shown that due to the impact of pollutants, as additional stressors, plant resistance against unfavourable natural factors had decreased plant tolerance limits to temperature, moisture, etc. [4-7]. On the other hand, an increasing number of investigations has shown the existence of cross-tolerance when exposure of plants to moderate stress factors induces resistance to other stresses [8, 9].

Important sources of Cd contamination are industry of non–ferrous metals, mining production, metal-contaminated wastes, application of pesticides and phosphate fertilizers [10-12]. Cadmium is one of the most toxic metals and due to its mobility it is easily absorbed by roots and can cause heavy damage to plants [11, 13]. Exposure to cadmium inhibits activity of photosynthetic enzymes, decreases chlorophyll content, increases membrane conductance and causes oxidative stress, resulting in inhibition of photosynthesis and growth [14-18].

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An important indicator which determines photosynthesis intensity is chlorophyll content in plant leaves. Cadmium markedly suppresses chlorophyll accumulation in leaves [19-22]. Carotenoid actively participates in photosynthesis as well and it was shown that content and ratio of carotenoids is strictly changed under impact of different stresses [23]. However, it has been determined that carotenoids are less sensitive to the impact of cadmium as compared to chlorophylls [24].

The negative impact of cadmium ions is usually modified by other factors [25]. A very important component of this integrated impact is soil acidity, which determines heavy metals content, mobility and the possibility to pass into the production system of a plant [26]. The highest amount of mobile cadmium is found in acidic soil, with pH values of 4.5-5.5 [27]. In such soils plants accumulate much more cadmium [28, 29].

Various experiments of fertilization in damaged forests confirm the proposition that additional nutrition increases plant resistance to various stresses [30]. Increased amounts of N, Ca, S and other elements decrease cadmium toxicity to the plants [29, 31]. Consequently, it could be possible to decrease a negative impact of anthropogenic pollution by supplementation of nutrients.

Taking in account that toxicity of heavy metals and other environmental pollutants involves different genotoxic mechanisms [32, 33], changes in some genetic indicators were investigated as well.

The main objective of this study was to estimate the impact of cadmium, substrate acidity and fertilization on the content of photosynthetic pigments, the mitotic index of cells and the water uptake indicators, as well as the disorder of genetic apparatus of *Lycopersicon esculentum* Mill.

Materials and Methods

Plant Material and Experimental Design

Experiments with the *Lycopersicon esculentum* Mill. (cv. Svara) were performed in growth chambers and vegetative platform of phytotron complex at the Lithuanian Institute of Horticulture in 2001-2002. The experiment lasted for 60 days.

Nine seedlings of *L. esculentum* with the first true leaves were transplanted to each vegetation pot (54×34×15 cm) to get a density of 50 plants m^{-2}. Transplanted seedlings were grown in phytotron chambers under illumination of SON-T Agro (Philips) lamps. Plants were exposed to a 14 h (day length) photoperiod and 24/17°C (day/night) temperature. The experiments were run in three replicates.

Six vegetation pots (20 l of substrate per unit) of each pH 4.5, pH 6.5 and pH 8.0 substrate acidity were prepared. For preparation of acidulated medium (pH 4.5) 2 l of solution with the addition of 60 ml H_2SO_4 and 30 ml HNO_3 were poured onto 20 l of peat substrate. For preparation of alkaline medium (pH 8.0), 20 l of peat substrate were admixed with 170 g of burnt lime (powdered CaO). Then pH values were determined. Untreated peat resulted in the reaction of pH 6.5.

Then three pots of each pH treatment were once watered with 2 l of 0.125 g l^{-1} cadmium sulphate crystalline [$3CdSO_4 \times 8H_2O$] solution to get a final Cd concentration of about 5.5 mg l^{-1}.

Subsequently, three fertilization variants of each Cd-pH treatment were prepared and studied in this experiment: NF – plants were not fertilized; F – 20 l of peat substrate were pre-fertilized by the addition of 10 g of fertilizers; 3F – 20 l of peat substrate were pre-fertilized by the addition of 30 g of fertilizers. Complex fertilizers "Kemira Ferticare NPK – 14:11:25 and microelements" (Kemira, Finland) were used for plant fertilization.

In addition, plants of F and 3F treatments were supplemented by nutrients (1 gl^{-1} (F) and 3 gl^{-1} (3F), respectively) once a week.

Extraction and Estimation of Chlorophylls and Carotenoids

The contents of carotenoids and chlorophylls *a* and *b* were measured in 100% acetone extract prepared according to the Wettstein method [34]. Measurements were done using spectrophotometers Spekol 11 (Carlzeiss Jena, Germany) (2001) and Genesys 6 (ThermoSpectronic, USA) (2002). At 60 days after planting (DAP) samples of *L. esculentum* for pigment extraction were taken from fully expanded canopy leaves.

Estimation of Stem Diameter and Sap Flow Rate

The phytometric system LPS-03 (PhyTech Ltd., Israel) was used for water exchange investigations on *L. esculentum* according to phytomonitoring methodology [35, 36]. The sensors were adjusted for the stem diameter variation and sap flow rate. Sensors were positioned on plants according to the recommendation of "PhyTech Ltd." [37, 38]. Monitoring with phytometric system LPS-03 was carried out in all fertilization and substrate acidity, except NF fertilization and pH 8.0 acidity variants as non-fertilized (NF) plants were not strong enough to bear a sensor and only two pairs of measurements could be run simultaneously. Measurements started as soon as plants were strong enough to bear sensors. Each process of measurement lasted for three days.

Determination of Mitotic Index

Macroscopic observations were made at 60 DAP. Some roots were cut and fixed in 95% ethanol: acetic acid (3:1), and 1-2 mm of the apical part of the root was squashed and stained with aceto-carmine. Mitotic index and chromosome aberrations were determined by 1000 anaphase-telophasis cells of root meristematic tissue in each variant.

The aberrations were classified in the following categories: bridges, fragments, chromosome elimination and other aberrations (mainly c-mitotic anaphases).

Statistical Analysis

All the results were presented as the means from 3 replications (plants). SD values were determined by MS Excel software and presented in all column charts as vertical bars.

Results

Analysis of chlorophyll content for non-fertilized variants showed that the amount of chlorophylls decreased in *L. esculentum* leaves while substrate alkalinity was increased. The highest content of chlorophyll was determined in leaves of *L. esculentum*, which grew in acidic medium at pH 4.5 (Fig. 1). Cadmium, in turn, caused a steep decrease of chlorophyll amount (approximately by 50%) in all acidity variants, but affected the variants at substrate acidity of pH 4.5 and pH 6.5 mostly.

Increased content of chlorophylls was observed under all pH values of cadmium-free F fertilization variant. However, the adverse cadmium effect decreased chlorophyll content in F plants down to the levels observed in non-fertilized (NF) cadmium-free samples. Content of chlorophylls in plants grown under F fertilization at pH 6.5 was reduced most of all.

A surplus of fertilizers (3F fertilization variant) markedly increased chlorophyll content in leaves of *L. esculentum* in pH 8.0 acidity variant – threefold as compared to NF variant. Plants of pH 4.5 and pH 6.5 acidity variants were less influenced. Content of chlorophylls in pH 4.5 variant even slightly decreased. The surplus of nutrients greatly inhibited an adverse effect of cadmium in plants grown under pH 6.5 (Fig.1).

Content of carotenoids in *L. esculentum* leaves has increased due to both F and 3F fertilization in all cadmium--free acidity variants (Fig. 2). Content of carotenoids in plants of 3F fertilization variant has increased roughly twice compared to non-fertilized variants. Adverse cadmium impact on content of carotenoids was not evident in any NF acidity variant. Such effect, indeed, decreased content of carotenoids in all acidity variants under 3F fertilization and in pH 6.5 acidity variant of F fertilization variant.

Patterns of stem diameter variation of *L. esculentum* at pH 4.5 substrate acidity were similar both in F and 3F fertilization variants (Fig. 3, a). Stem diameter of F fertilized plants increased more rapidly compared to 3F fertilization variants, likely because of higher turgor of stem cells. The growth of stem diameter slowed down under the impact of cadmium (Fig. 3, b). At F fertilization variant positive daily stem evolution was determined even under the influence of cadmium.

3F amount of fertilizers greatly stimulated an increase in stem diameter of samples grown under pH 6.5 (Fig. 4), though adverse effect of cadmium was greater as well. Cadmium impacts on F fertilized samples were less obvious as compared to 3F fertilization variants.

A higher relative sap flow rate was determined for 3F-fertilized *L. esculentum* of acid (pH 4.5) substrate variant as compared to F-fertilized samples (Fig 5, a). Relative sap flow rate of 3F-fertilized plants was slowed under the effect of cadmium. However, it remained stable in F-fertilized plants, though it was lower compared to cadmium-free samples (Fig. 5, b).

Under pH 6.5 relative sap flow rate was higher in 3F-fertilized *L. esculentum* as compared to F-fertilized plants (Fig 6, a). Relative sap flow rate of F-fertilized plants remained stable, though it had a tendency to decrease in 3F-fertilization samples. Cadmium suppressed relative sap flow rate in all fertilization variants, especially in 3F-plants (Fig. 6, b).

Mitotic index of *L. esculentum* root cells had a tendency to increase respectively to ascending substrate pH

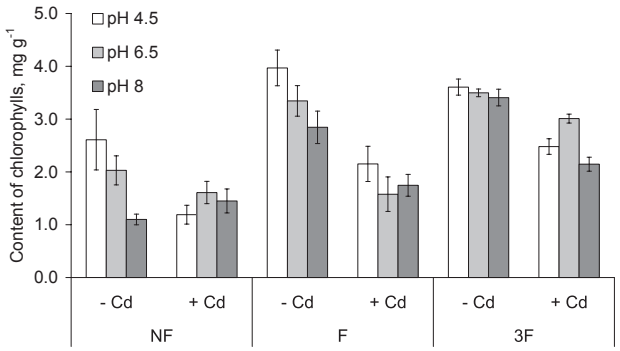

Fig. 1. Chlorophylls content in leaves of *Lycopersicon esculentum* Mill. under different acidity and nutrition (NF – plants were not fertilized; F – 20 l of peat substrate were pre-fertilized by the addition of 10 g of fertilizers; 3F – 20 l of peat substrate were pre-fertilized by the addition of 30 g of fertilizers) at 60 DAP.

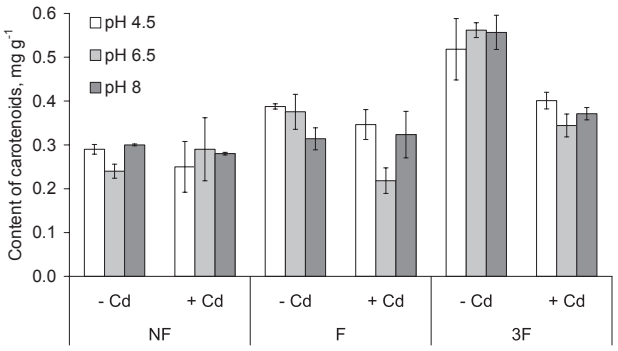

Fig. 2. Carotenoid content in leaves of *Lycopersicon esculentum* Mill. under different acidity and nutrition (NF – plants were not fertilized; F – 20 l of peat substrate were pre-fertilized by the addition of 10 g of fertilizers; 3F – 20 l of peat substrate were pre-fertilized by the addition of 30 g of fertilizers) at 60 DAP.

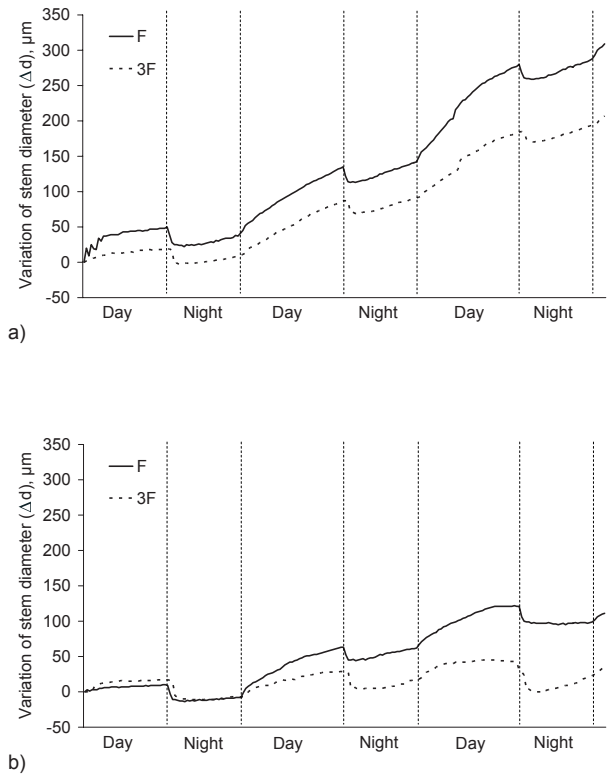

Fig. 3. Stem diameter variation of *Lycopersicon esculentum* Mill. under pH 4.5 and different nutrition without (a) and with (b) cadmium supplementation (F – 20 l of peat substrate were pre-fertilized by the addition of 10 g of fertilizers; 3F – 20 l of peat substrate were pre-fertilized by the addition of 30 g of fertilizers). Measurements were started at 35 DAP.

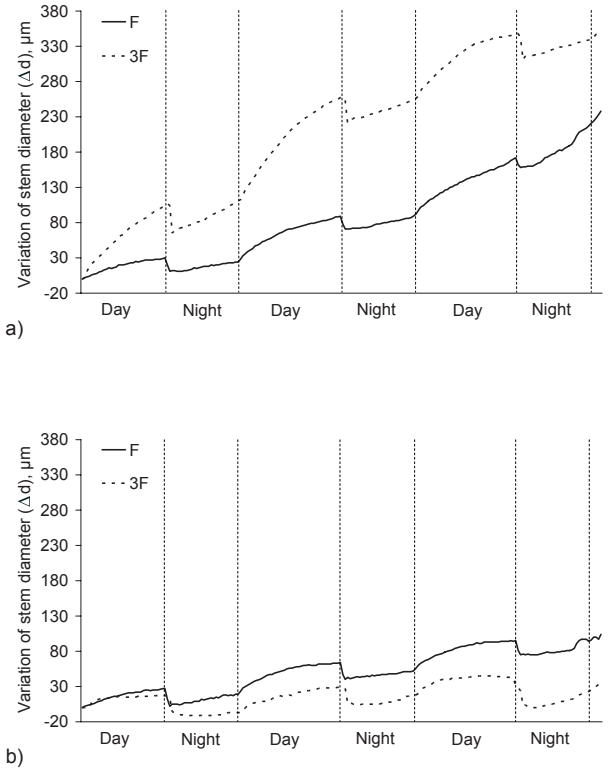

Fig. 4. Stem diameter variation of *Lycopersicon esculentum* Mill. under pH 6.5 and different nutrition without (a) and with (b) cadmium supplementation (F – 20 l of peat substrate were pre-fertilized by the addition of 10 g of fertilizers; 3F – 20 l of peat substrate were pre-fertilized by the addition of 30 g of fertilizers). Measurements were started at 35 DAP.

value. However, a statistically significant adverse effect of cadmium on mitotic index was not evidenced in any fertilization variant, except for 3F plants grown under acidic (pH 4.5) conditions (Fig. 7).

The mitosis with anomalies (chromosome breaks, fragmentation, bridges, chromosome eliminations and abnormal nucleus divisions) was noticed under the impact of cadmium in *L. esculentum* root cells (Fig. 8).

Discussion

In general, content of photosynthetic pigment, especially chlorophyll, decreased in response to the toxic impact of cadmium at various substrate pH values. This effect was the most evident in acidic substrate. F and 3F fertilization rates increased resistance of chlorophylls to the adverse effect of cadmium (Fig. 1).

Stem diameter variation and relative sap flow rate are important indices of plant water uptake. They also can be used as indirect indices of transpiration intensity. Relationships between balanced leaf temperature, which reflects the process of transpiration, stem diameter which shows turgor of stem cells, and the relative sap flow rate determine functional correlation and normal run of such integral processes as mineral nutrition, photosynthesis, respiration, transportation and metabolism of photosynthetic produce [36].

Studies on *L. esculentum* revealed that 3F amount of fertilizers suppressed variation of stem diameter, especially in pH 4.5 acidity variant (Fig. 3, a). Thus, turgor of stem cells was decreased. Greater relative sap flow rate was observed in 3F fertilized plants as compared to F fertilized plants (Fig 5, a). This suggests that plants easily transpirated but, probably, the process of evaporation was inadequate to cool leaves, which were warmed due to intensive metabolism processes. Stem diameter increment was not noticed (Fig. 3, b) and relative sap flow rate weakened daily (Fig. 5, b) in 3F-fertilized cadmium affected plants grown in acidic substrate (pH 4.5).

Substrate acidity of pH 6.5 under 3F fertilization stimulated the processes of water exchange, hence, stem diameter increment (Fig. 4, a) and relative sap flow rate (Fig. 6, a). At this point 3F fertilization only stimulated these processes and did not provoke negative subsequence in transpiration and turgor. However, cadmium, as observed in pH 4.5 substrate acidity samples, inhibited these processes (Fig. 4 and 6, b), especially in com-

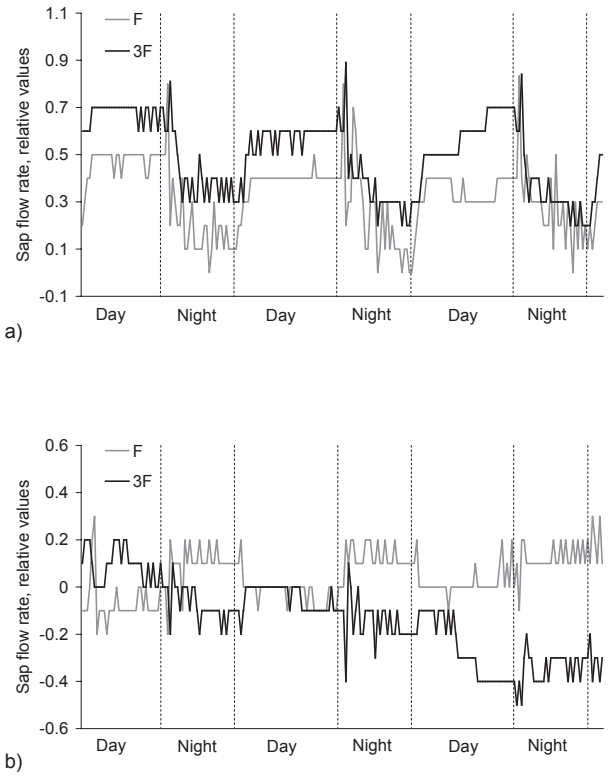

Fig. 5. Relative sap flow rate of *Lycopersicon esculentum* Mill. under pH 4.5 and different nutrition without (a) and with (b) cadmium supplementation (F – 20 l of peat substrate were pre-fertilized by the addition of 10 g of fertilizers; 3F – 20 l of peat substrate were pre-fertilized by the addition of 30 g of fertilizers). Measurements were started at 35 DAP.

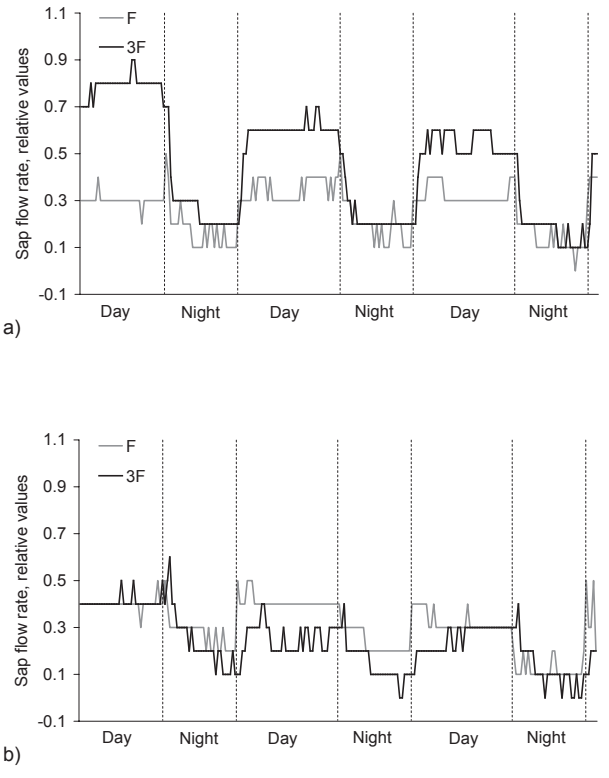

Fig. 6. Relative sap flow rate of *Lycopersicon esculentum* Mill. under pH 6.5 and different nutrition without (a) and with (b) cadmium supplementation (F – 20 l of peat substrate were pre-fertilized by the addition of 10 g of fertilizers; 3F – 20 l of peat substrate were pre-fertilized by the addition of 30 g of fertilizers). Measurements were started at 35 DAP.

bination with a high amount of biogens. Thus, processes of water exchange, transpiration and turgor maintenance in such plants were disturbed. Consequently, stress-related disbalance of plant function is inspired [36]. These findings imply that cadmium affects not only the transpiration of *L. esculentum* under such conditions. Furthermore, downgraded range of plant tolerance under a few stresses, including cadmium, was noticed for other physiological indices such as organogenesis, amount of photosynthetic pigments, and changes of proline content [25, 39, 40].

As shown in Fig. 7, the mitotic index did not vary according to different substrate pH levels, though cadmium in all fertilization variants of high (pH 4.5) substrate acidity tended to decrease the mitotic index. Changes of cell mitotic index occurred due to the disorder of genetic apparatus under the impact of cadmium (or along with other anthropogenic factors). Such disorder was evidenced by chromosome breaks, fragmentation, bridges, chromosome eliminations and abnormal nucleus divisions (Fig. 8). In most cases a common trend was noticed – the more intensively plant cell division proceeded the more evident was the toxic effect of cadmium. Sensitivity of the genetic apparatus of various plants to the toxicity of cadmium was dependent on substrate acidity and amount of nutrients as well.

The general conclusion can be made that nutrient deficiency leads to evident plant growth retardation, and higher nutrient favours plant growth under the effect of cadmium.

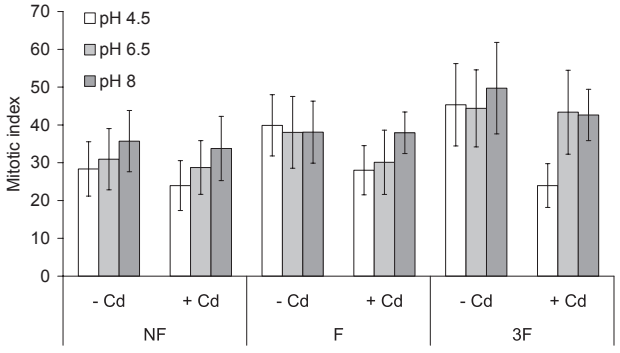

Fig. 7. Mitotic index in roots of *Lycopersicon esculentum* Mill. under different acidity, nutrition and supplementation of cadmium (NF – without fertilizers, F – 10 g fertilizers / 10 l water, 3F - 30 g fertilizers / 10 l water).

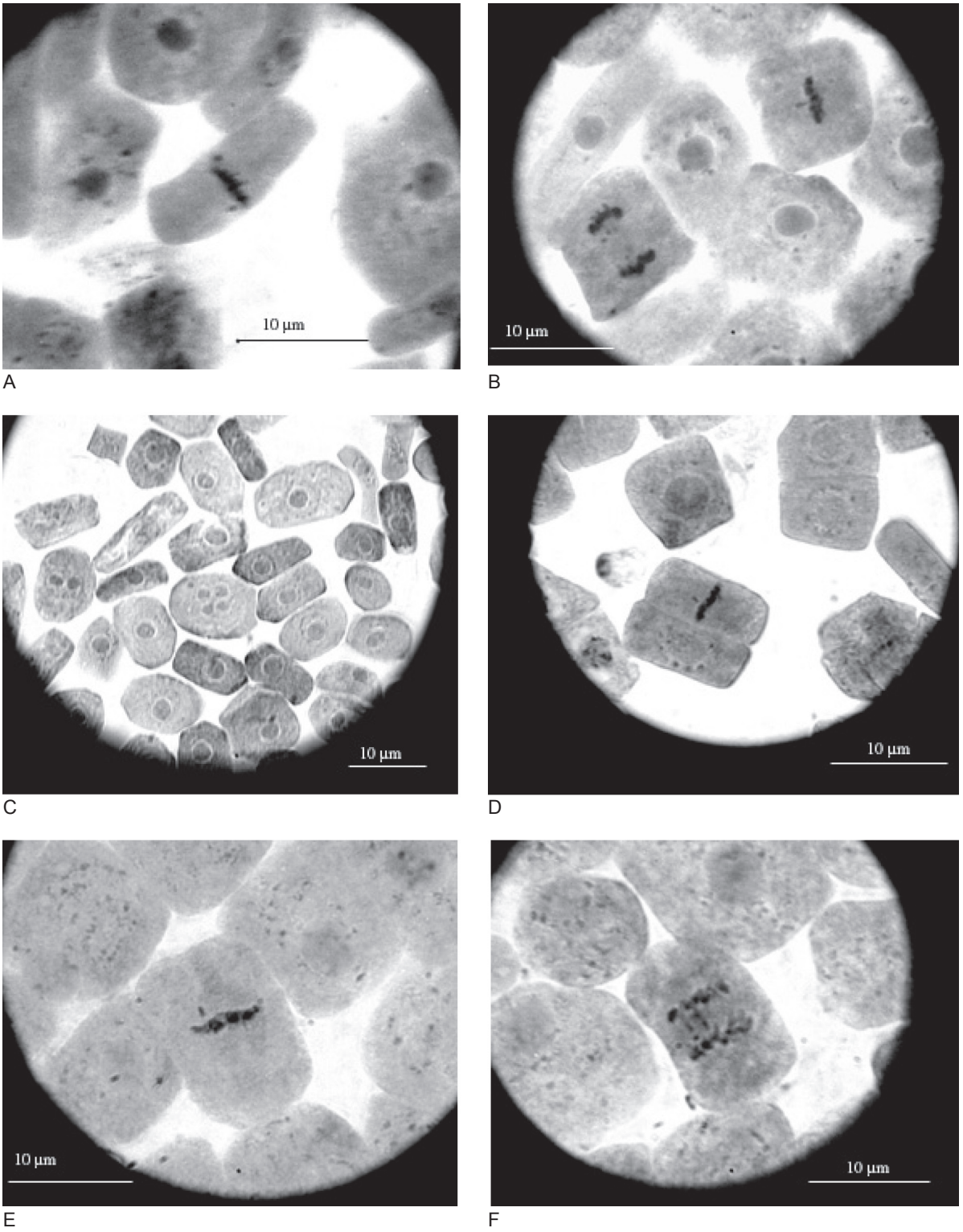

Fig. 8. The mitosis of *Lycopersicon esculentum* Mill. with anomalies. (A, D, E – chromosomes eliminations, B – chromosomes fragmentation, C – abnormal nucleus divisions, F – bridge); all root samples were obtained from Cd-treated plants grown under pH 4.5, where A, D and E plants were fertilized (F fertilization variant) and B, C and F were not fertilized (NF plants).

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