A DNA cytophotometric study of salt adaptation in *Allium cepa* and *Nicotiana bigelovii*

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Abstract — Nuclear DNA was measured by Feulgen cytophotometry in NaCl-exposed *Allium cepa* roots and *Nicotiana bigelovii* calli cultured *in vitro*. Analysis of the differentiation zone of *A. cepa* roots grown in water showed that only 2.8% of the nuclei had DNA contents higher than 4 C, where 2 C nuclei were predominant. In roots grown in salt water, 2 C nuclei were less numerous than 4 C nuclei and the DNA content was greater than 4 C in 19.2% of the cells, with 1.4% of the cells having a DNA content of 16 C. Two C, 4 C, 8 C, and a few 16 C nuclei, together with many nuclei with intermediate DNA content values, occurred in both the control and salt-treated calli of *N. bigelovii*. About 49.1% of the nuclei had DNA values around 2 C in the controls, while the percentage of cells with nuclei with higher DNA content values increased in calli as the NaCl concentration in the medium increased. Fifteen per cent of the nuclei had DNA values around 16 C in calli grown on media with 10 gr l^{-1} NaCl added, compared to 2.8% in the controls. In calli exposed to 15 gr l^{-1} NaCl, 18.4% of the nuclei had DNA contents around 16 C, and 3.1% of the nuclei had a DNA value of 32 C. These results are added evidence that chromosome endoreduplication occurs in plants as a widespread adaptive response to salt.

Key words: *Allium cepa*, endopolyploidy, Feulgen cytophotometry, *Nicotiana bigelovii,* salt adaptation.

INTRODUCTION

Chromosome endoreduplication (endopolyploidy) is a well known phenomenon by which the DNA content in the cell nucleus increases geometrically through repeated DNA synthesis that is not followed by mitosis. Endopolyploidy, which may lead to a very high DNA content in the nucleus (*e. g.*, some cells in the embryo suspensor of *Phaseolus coccineus* may reach 8,192 C DNA; Brady 1973), is related to cell enlargement and accompanies cell differentiation in many animal and plant tissues (D'amato 1952).

Endopoyploidy has also been observed in tissues of plants that had been exposed to salinity. Catarino (1965; 1968) found that sea water treatments induced endoreduplication followed by cell enlargement in root cortex cells of *Lobularia maritima* and *Bryophyllum crenatum*. More recently, Ceccarelli *et al*. (2006) found chromosome endoreduplication in root cortex cells of *Sorghum bicolor* cv. 610 plants that had been exposed to sublethal salinity level (150 mM NaCl) during their early development. This treatment gave the plants the capacity to grow and set seeds at a NaCl concentration of 300 mM (Amzallag *et al*. 1990). In contrast, in *S. bicolor* plants which have another genotype (DK 34-Alabama), the DNA content in the root cortex cells did not increase in response to the initial NaCl-exposition, and these plants did not survive on the salt-containing substratum.

These observations suggest a stringent correlation between chromosome endoreduplication in certain tissues and salt adaptation, and raise a question about the general occurrence of this adaptive response in plants. In an attempt to shed some light on this intriguing question which is of importance at both the theoretical and applied levels, a study was carried out to determine if ex-

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posure to salt induces endopolyploidy in tissues of two other plant species under different developmental conditions. A cytophotometric study was carried out on the nuclear DNA content in cells of habituated calli from seedlings of *Nicotiana bigelovii* grown *in vitro* in the presence of high NaCl concentrations and in roots of *Allium cepa* bulbs that were grown in salt water. The *in vitro* system of *N. bigelovii* (BENNICI *et al.* 1972; BENNICI and CAFFARO 1985) was selected because it is a more simplified system than a whole plant organ and it excludes any possible interference of exogenous growth regulators. The system of *A. cepa* roots is a very simple *in vivo* test by which the response to salt can be studied during a developmental event. Moreover, the root tip is often the first part of any plant which is likely to come into contact with chemicals present in soil and water.

MATERIALS AND METHODS

Plant material - Nicotiana bigelovii var. *bigelovii* seeds were surface-sterilized by immersion in a 20% aqueous solution of sodium hypochlorite (7% active chlorine) for 20 min. After four washes in sterile distilled water, the seeds were placed in Petri dishes containing MURASHIGE and SKOOG's (1962) basal medium (MS) supplemented with 0.4 mg l^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D) and 30 g l^{-1} sucrose, and solidified with 0.8% Difco Bacto agar. Calli formed at the hypocotyl/root transition zone of germinated seeds were transferred to 100 ml Erlenmeyer flasks containing 30 ml solid MS medium devoid of 2.4-D and subcultured for 6 months (a transplant every 20 d) to ascertain the acquisition of the habituation capacity. After having determined the NaCl toxicity threshold in three subcultures on MS media as described above to which 5-25 gr l^{-1} NaCl had been added, the calli were cultured for 40 d (two subcultures) on MS minimal media containing 10 and 15 gr l^{-1} NaCl (20 calli for treatment), respectively.

All culture containers were kept in a growth room at 25 ± 1°C with 16 h day/8 h night photoperiod under fluorescent light (35 µmol m^{-2} s^{-1}).

After preliminary tests of salt toxicity using NaCl concentrations from 2.5 to 10 g l^{-1}, ten bulbs of *Allium cepa* cv fiorentina, grown in the field without any treatment, were placed in vessels (the classical *Allium* test; FISKESJO 1985) containing distilled water to which 5 gr l^{-1} NaCl was added, and the roots were allowed to grow for 4-5 d to a length of 4-6 mm (the liquid being changed every day). The onions bulbs were kept in the growth chamber as above reported. Control materials were obtained by growing bulbs or calli under the same respective above-mentioned conditions but in medium or water without salt.

Materials to be used for DNA cytophotometry were fixed in ethanol-acetic acid 3:1 (v/v). Small portions of calli grown for 30 d in presence of the salt and control were chosen at random from each callus, while 5 root tip samples, chosen at random, were collected from each onion bulb.

DNA cytophotometry - After treating portions of fixed roots or calli with a 5% aqueous solution of pectinase (Sigma) for 45 min at 37°C, they were squashed under a coverslip in a drop of 45% acetic acid. The coverslips were removed by the solid CO_2 method and the preparations were Feulgen-stained after hydrolysis in 1 N HCl at 60°C for 8 min. After staining, the slides were subjected to three 10 min washes in SO_2 water prior to being dehydrated and mounted in distyrene dibutylphthalate xylene (DPX; BDH Chemicals). Since simultaneous processing was not possible due to the large number of preparations to be analyzed, squashes made with the root tips from a single *A. cepa* bulb were concurrently stained with each group of slides and used as standards in order to make all the results comparable.

Feulgen DNA absorptions in individual cell nuclei were measured at a wavelength of 550 nm using a leitz MPV3 microscope photometer equipped with a mirror scanner. DNA C values were calculated from the absorption values of early prophases (= 4C).

RESULTS AND DISCUSSION

The DNA content of cell nuclei in the apex and differentiation zone of *A. cepa* roots grown in 5 gr l^{-1} NaCl and those of the control roots are reported in Fig. 1. In the root apex, the distribution of Feulgen absorptions was typical of a population of actively proliferating cells. The results were similar in the control and salt-treated materials, except for a few nuclei (6.2%) that had DNA contents higher than 4 C, which were only found in the apex of salt-treated roots. In contrast, noticeable differences were found between the control and salt-treated roots when the differentiation zone was analyzed. A few nuclei (2.8%) had DNA contents higher than 4 C in the controls, where 2 C nuclei were predominant. In roots grown in

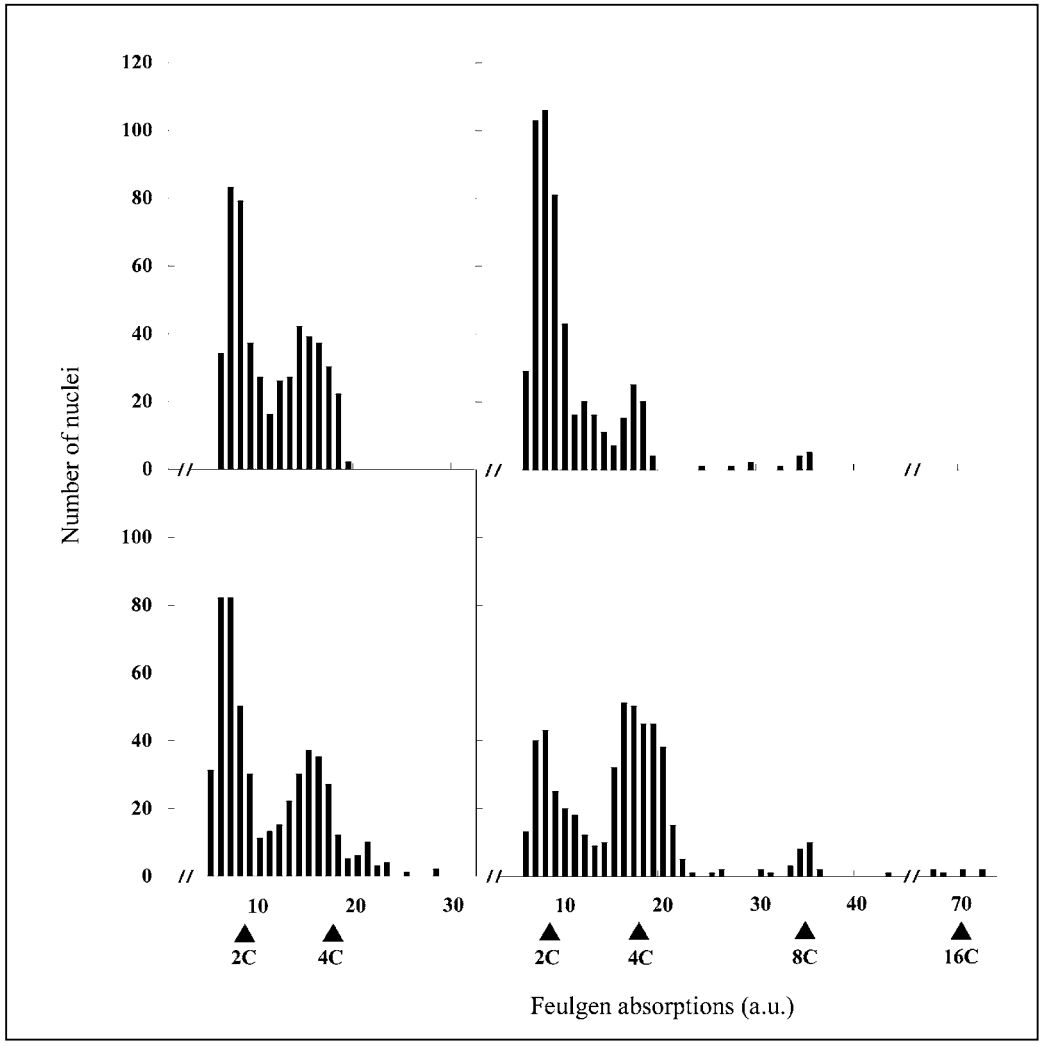

Fig. 1 — Feulgen absorptions of cell nuclei in the apex (*left*) and the differentiation zone (*right*) of control (*top*) and salt-exposed (*bottom*) *Allium cepa* roots. One-hundred randomized nuclei in each portion of five roots from five bulbs were analyzed.

salt water, there were fewer 2 C nuclei than 4 C nuclei, and the DNA content was higher than 4 C in a consistent number of cells (19.2%), some of which (1.4%) had a DNA content as high as 16 C. By analyzing the vascular cylinder and the outer tissues (cortex and epidermis) of *S. bicolor* roots separately, it was shown that an increase in nuclear DNA contents in salt-treated plants was confined to cortex and epidermal cells (CECCARELLI *et al.* 2006). In contrast, a similar analysis in salt-treated roots of *Allium cepa* showed that most of the cells that had DNA contents higher than 4 C were located in the vascular cylinder (Fig. 2). These results indicate that there is tissue specificity in the response to salt as well as species-specific tissue competence for salt adaptation.

The results given in Fig. 3 show the effect of adding NaCl to the medium in *N. bigelovii* callus cultures. Two C, 4 C, 8C, and a few 16 C nuclei, together with many nuclei with intermediate DNA content values were found in the control calli. Similar Feulgen DNA values were found in salt-treated calli, but the proportion of nuclei with higher DNA contents increased markedly as the NaCl concentration in the medium increased. The relative proportions of nuclei grouped according to their Feulgen absorption values are reported (Fig.4). Nuclei with the lowest absorptions (DNA values around 2 C) were prevalent (49.1%) in the control calli. The proportion of nuclei with higher DNA content clearly increased in calli grown on the medium to which 10 gr l^{-1}

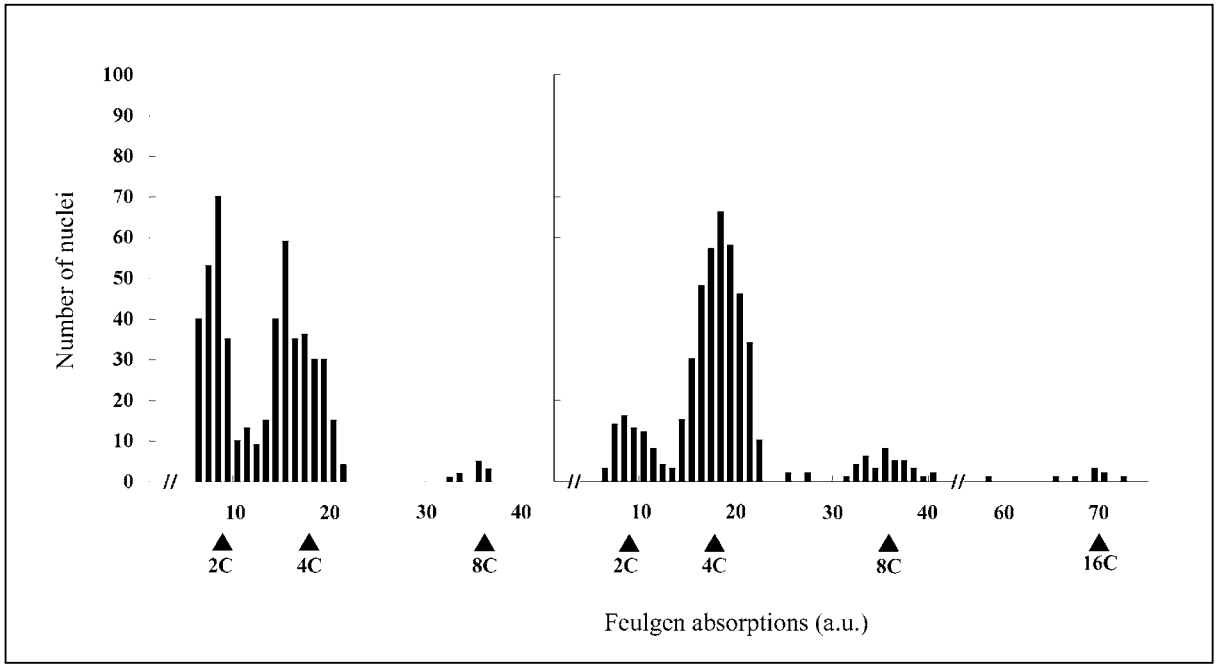

Fig. 2 — Feulgen absorptions of cell nuclei from different anatomical portions of the differentiation zone of salt-exposed roots of *Allium cepa*. *Left*, cortex and epidermis. *Right*, vascular cylinder. One-hundred randomized nuclei in each anatomical region of five roots from five bulbs were analyzed.

NaCl had been added; 15% of all the nuclei measured had DNA values around 16 C compared to 2.8 % in the control calli. This trend became even more marked in calli exposed to 15 gr l^{-1} NaCl, where 18.4% of the nuclei had DNA contents around 16 C and 3.1% of the nuclei had DNA values around 32 C.

As already observed in *L. maritima*, *B. crenatum* and *S. bicolor* (CATARINO 1965 1968; CECCARELLI *et al.* 2006), the results of this study show that endopolyploidy is involved in salt treated materials. It is known that chromosome endoreduplication is a factor of cell differentiation (D'AMATO 1952) and that endoreduplicated cells, which are generally found in plant tissues that are grown *in vitro* (BENNICI 2004), occur in habituated calli of *N. bigelovii* (BENNICI and CAFFARO 1985). In fact, it can be seen (Figs. 1 and 3) that endopolyploidy occurs, to a limited extent, in the controls. Therefore, the greater number of endopolyploid cells that were found in the materials treated with salt might simply have been due to selection in favour of root and callus cells in which chromosome endoreduplication had occurred to a certain extent, independent of salt exposure. However, the endopolyploidy levels found in the controls were not as high as in the salt-treated roots or calli (Figs. 1 and 3). It was also observed that the response of *A. cepa* roots to salt was tissue-specific. A similar observation was made in *S. bicolor* by CECCARELLI *et al.* (2006); these authors also found that not all genotypes were competent for NaCl-induced endopolyploidy. These observations seem to contradict the selection hypothesis. The data suggest that NaCl can actively induce endopolyploidy in plant cells and that chromosome endoreduplication is a part of the adaptive response to salinity. Endopolyploidy may be a way to modulate gene expression (SOLTIS and SOLTIS 2000; PIKAARD 2001) without having recourse to other adaptive reorganization events in the genome such as extensive extrasynthesis or underrepresentation of repeated DNA sequences, which have been shown to take place in different plant species in response to a stressful environment (reviewed by CULLIS 1999; CIONINI 2004).

The results of this study allow two systematically distant species to be added to the list of plant species in which endopolyploidy occurs in response to salt exposure (CATARINO 1965; 1968; CECCARELLI *et al.* 2006). Moreover, the results obtained in *N. bigelovii* calli prove that this response can also occur in cells that are not inserted in organized tissues. Therefore, chromosome endore-

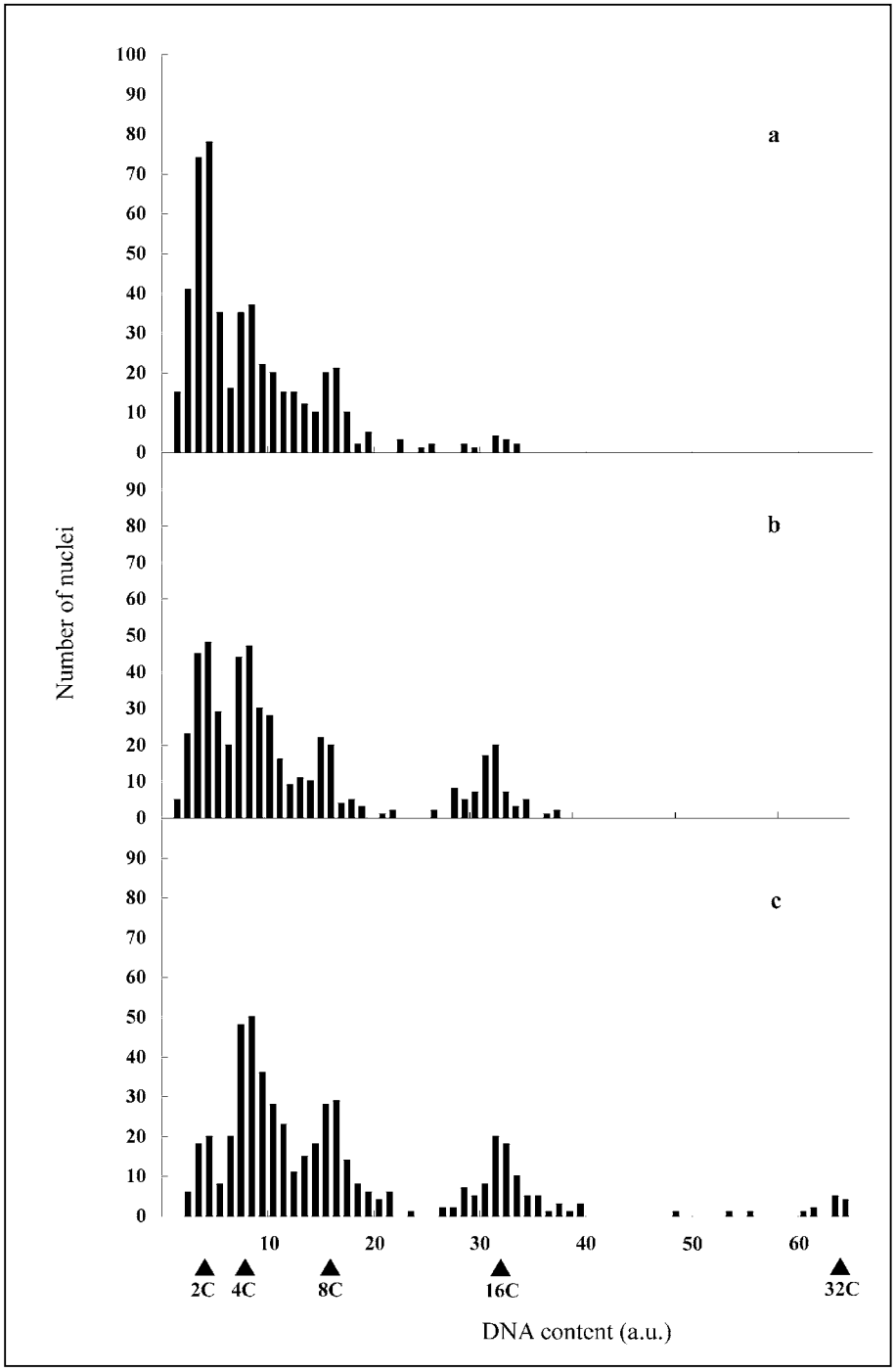

Fig. 3 — Feulgen absorptions of cell nuclei in *Nicotiana bigelovii* calli grown on salt-free medium (a) or on media to which 10 gr l^{-1} NaCl (b) or 15 gr l^{-1} NaCl (c) were added. One-hundred randomized nuclei in each of five explants for each medium were analyzed.

duplication, that allows plant cells to survive and grow in the presence of NaCl in the substratum, seem to be an adaptive response of widespread occurrence in plants.

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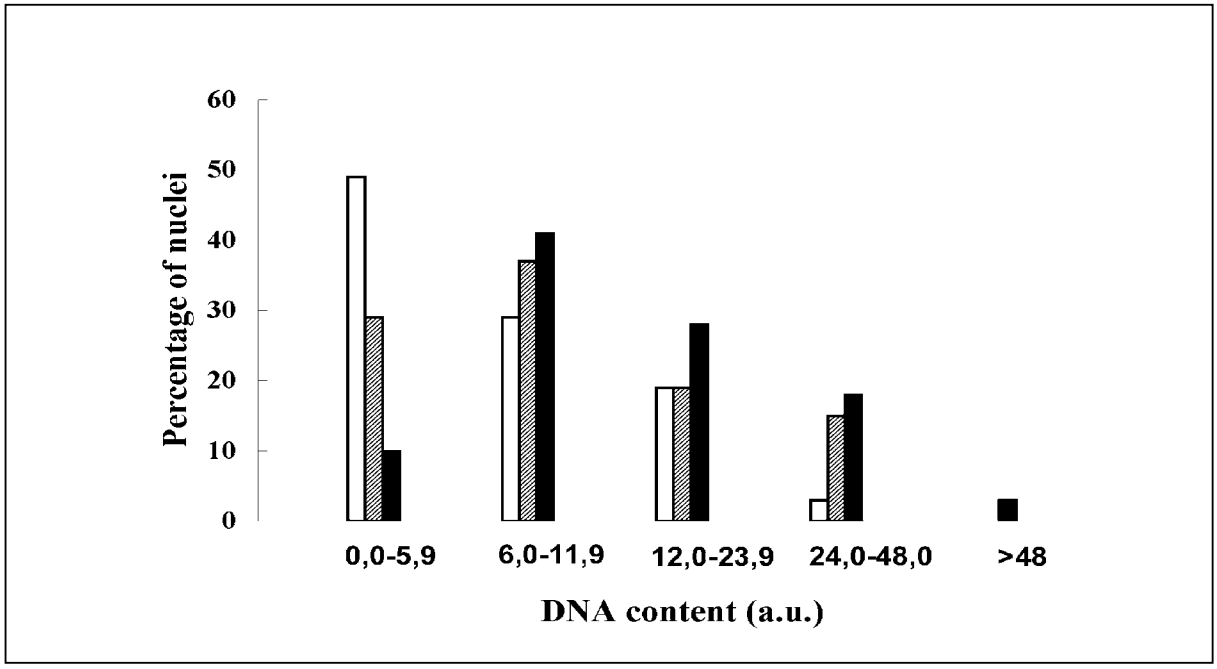

Fig. 4 — Percentage of nuclei with different Feulgen absorptions in *Nicotiana bigelovii* calli grown on salt-free medium (open bars) or on media to which 10 gr l^{-1} NaCl (broken bars) or 15 gr l^{-1} NaCl (solid bars) were added. Data were drawn from Feulgen absorptions in Fig. 3.

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