changing environment and may thus be of importance for plant improvement.

*Abbreviations: Pi = Na2HP04/KH2P04.

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Acetaldehyde induces mature endoreduplicated *Allium cepa* root cells to divide

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Summary. In *Allium cepa* root tips treated with acetaldehyde, metaphase cells showing diplochromosomes are occasionally observed. The short treatment time excludes the possibility that the endoreduplication has been induced by the chemical. Instead, it seems that acetaldehyde is able to stimulate, directly or indirectly, the division of mature cells previously endoreduplicated.
Key words. Acetaldehyde; endopolyploid cells; diplochromosomes.

In most higher plants, both monocotyledons and dicotyledons, the cells of mature root tissues, in their process of development, undergo chromosome doubling by endomitotic reduplication without subsequent mitosis[1-3]. This chromosome doubling in differentiated tissues was first reported after stimulation of these mature cells to divide by wounding[4] or by treatment with plant hormones, namely auxins[5] or kinetin[6]. In all these cases, it has been well established that the endomitotic reduplication had occurred prior to these treatments rather than being induced by the experimental procedure itself.

On the other hand, cells showing diplochromosomes, i.e., chromosomes made up of four chromatids, are frequently observed among the polyploid mitoses which are characteristic of the nodular tissues of leguminosae after inoculation with *Rhizobium leguminosarum*[7]. Nevertheless, in spite of the extensive studies carried out, the internal molecular mechanisms which control these cells, preventing them from dividing under normal conditions, are poorly understood to date.

Acetaldehyde, the first metabolite of ethanol, has been reported to be clastogenic (able to break chromosomes) and to induce sister chromatid exchange[8]. In a study performed by us in order to evaluate the effectiveness of this chemical with regard to its cytotoxicity and genotoxicity in *Allium cepa* root tip cells[9], metaphase cells showing diplochromosomes were occasionally observed. In this plant material, Hervas[10] has reported the mitotic activity of endopolyploid cells after a pulse treatment with excess thymidine.

The aim of the present study was to try to determine, for a short-term experimental schedule, the effectiveness of both acetaldehyde and thymidine in inducing endoreduplicated cells to divide. The short treatment time in acetaldehyde was chosen because of the high toxicity of this chemical.

Materials and methods. The material consisted of root meristems of *A. cepa*. The onion bulbs, 15–30 g in weight, were grown in the dark at constant temperature (20 °C) with tap water renewed every 24 h. All the treatments began when the roots were 15–20 mm in length.

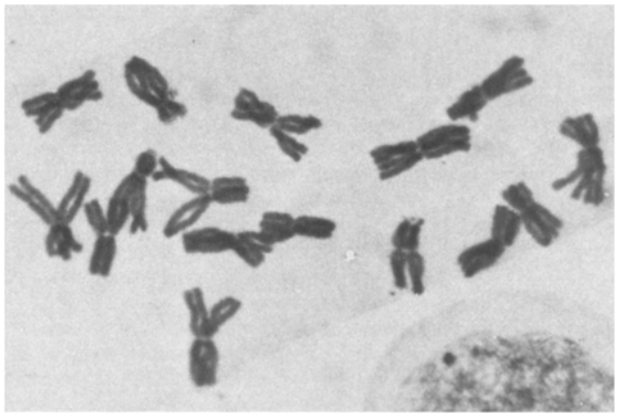
Roots, still attached to the bulbs, were treated with acetaldehyde (Merck) dissolved in distilled water at different concentrations (0.05; 0.1 and 0.2%) for 2 h or with a solution of thymidine (Fluka) in tap water at a concentration of 5 mg/ml for 10 min followed by either a treatment with 0.05% colchicine in distilled water for 2 h, or with 0.05% acetaldehyde for the same period. After treatments, root tips were fixed in mixture of methanol: acetic acid (3:1) at 5 °C overnight and prepared as Feulgen squashes.

Results and discussion. No metaphase cells showing diplochromosomes were observed after a 2-h-treatment with 0.05% colchicine. Both for 0.05 and 0.1% acetaldehyde, a strong C-

Effectiveness of short-term treatments with acetaldehyde and thymidine in the induction of endopolyploid mitosis in *A. cepa* root tips

Agent	Dose	Post-treatment	Normal mitosis per meristem (mean ± SEM)[a]	Endopolyploid mitosis (mean ± SEM)
Colchicine	0.05%	–	1.157.6 ± 12.2	0
Acetaldehyde	0.05%	–	356.0 ± 6.7	0
	0.1%	–	446.0 ± 7.5	0.3 ± 0.2
	0.2%	–	n.m.	n.m.
Thymidine	5 mg/ml	0.05% Colch.	934.3 ± 10.9	0
	5 mg/ml	0.05% Acetald.	526.2 ± 8.2	0.06 ± 0.1

[a] 30 root tips, 6 from each of 5 bulbs were analyzed in all cases. n.m., no mitosis.



Endopolyploid root tip cell of *A. cepa* arrested in metaphase after a 2-h-treatment with 0.1% acetaldehyde, showing the typical diplochromosomes.

mitotic effect (arresting of the cells at metaphase) was evident. After the 2-h-treatment, no normal anaphases were observed. The highest dose employed (0.2%), resulted in a complete inhibition of mitosis (table). A 2-h-treatment with 0.1% acetaldehyde resulted in the highest frequency of endoreduplicated cells in mitosis (table and fig.), although, even so, the percentage of cells showing diplochromosomes was extremely low. A lengthening of the treatment time did not yield an increase in the frequency of endoreduplicated cells in mitosis but proved to be very toxic. The short treatment time rules out the possibility that such a treatment with acetaldehyde should have induced the endoreduplication of these cells. Instead, it seems that this chemical is able to stimulate spontaneous endopolyploid cells previously occurring in the meristem to divide.

In order to compare the short-term effect of acetaldehyde with that reported for excess thymidine[10], the roots were placed in colchicine for 2 h after a treatment with excess thymidine for 10 min. No endopolyploid mitosis was observed (table) indicating that a posttreatment of at least 3–5 h seems to be necessary for thymidine-stimulated cells to enter mitosis[10]. Nevertheless, when the treatment with thymidine was followed by a 2-h-posttreatment with 0.05% acetaldehyde, that per se did not induce endopolyploid mitosis, 2 cells showing diplochromosomes were observed (table). The extremely low number of endopolyploid cells in mitosis found by us is not surprising, if we consider that these are only a subset of the differentiated cells or the cells undergoing a process of differentiation, and therefore, their frequency in root tips cannot be expected to be high.

Since acetaldehyde has been shown to be a strong antimitotic agent in *A. cepa* root tip cells[9], it is rather surprising that a substance of this high toxicity could be able to stimulate mature cells to enter mitosis. Both auxins[5] and Kinetin[6] have been reported to act as triggers for mitosis in mature endomitotic plant cells. It has been also proposed that rhizobial infection might be analogous to the cytokinin-auxin stimulation, causing endomitotic cortical cells already existing in the root to undergo division and initiate nodules[7,11]. On this basis, the simplest explanation for the induction of endopolyploid mitosis by acetaldehyde appears to be that this chemical is able to induce a hormonal imbalance in the root. Nevertheless, a direct action at the cellular level to stimulate mitosis of mature cells cannot be excluded.

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Penicillium auxotrophic mutants can be detected by using xanthene dyes

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Summary. Auxotrophic mutants of *Penicillium* spp. have been directly isolated after mutagenic treatment from agar plates containing Xanthene dyes. They grow as characteristic small colored colonies. Some strains were tested and they showed a differential response depending on the Xanthene dye used.
Key words. Penicillium; Xanthene dyes; auxotrophs, selection of.

The dye Phloxin B (tetrabromotetrachlorofluorescein, Magdala Red) has been used to facilitate the detection of yeast mutants[1–4]. A common response of all of these mutants (respiratory-deficient[1], auxotrophic[2,3] and nitrogen-utilization mutants[4]) was the production of intensely stained small colonies that could be visually selected. In a previously described attempt to use this method with a filamentous fungus *(Aspergillus nidulans)*, auxotrophs and prototrophs were not clearly distinguished, but Magdala Red induced paramorphic colony growth of *A. nidulans* and *Penicillium* spp[5]. Of various Magdala Reds investigated by Scott[6], only Phloxin B induced paramorphic colonies in *Neurospora crassa*. Prompted by these results, we have examined the effects of Phloxin B, Eosin B and Eosin Y with *Penicillium* spp., and we have found that the dyes could indeed be used for the visual selection of auxotrophic mutants of these fungi. Xanthene dyes are used to color food and in hair treatment preparations. However, opinions differ as to whether these dyes are mutagenic[7]. We have also addressed this question here, and we have not detected mutagenicity or any effect on viability under our conditions.