the control cells – which had not been previously exposed to exogenous digoxine – was 4.3 ng/100,000 cells (fig.). Taking into consideration that the therapeutic dose level of digoxin is 0.8–4.5 ng/ml of sera for humans, the concentration of digoxin or immunologically demonstrable digoxin-like materials (the kit used also detects digitoxin and dihydro-digoxin) in *Tetrahymena* is appreciable.

We performed pretreatment with digoxin or the related glycoside ouabain on the ground that the glycosides may stimulate their own synthesis under certain conditions[11]. The quantitative relations of uptake differed considerably between digoxin- or ouabaine-pretreated and control cells, but statistical evaluation was hampered by extreme intraassay variations. The marked, although not significant increase in the digoxin content of the digoxin-pretreated cells might have been due to intracellular retention of part of the considerable amount of digoxin used for preexposure. This could be ruled out in the case of ouabain, since the cross reaction of the kit used with ouabain was only 0.029% of its reaction with digoxin, i.e. it did not appreciably differ from the control.

The precise role of endogenous digoxin for *Tetrahymena* is at the time being obscure, but so are the role of ACTH, insulin, somatostatin and relaxin. The authors[6–10] who detected the latter hormones in *Tetrahymena* have postulated that after release from the cell, the hormones are involved in the control of receptor activity by a feedback mechanism. We failed to find evidence of this in earlier studies[12] or in the present experiments. Digoxin does not occur in man, except when administered as a drug, and its natural occurrence in other animals is exceedingly rare (e.g. in toad poison)[13] – what might then be its biological role in *Tetrahymena*? It seems very likely that the lowest levels of phylogenesis represent an 'experimental stage' in respect of the biosynthesis of polypeptides and steroids (like tetrahymanol) and the byproducts or intermediary products of such biosynthetic processes could result in the formation of hormones and of digoxin-like molecules as well.

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Somatic variation in micropropagated clones of *Cichorium intybus*

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Summary. An experiment aiming to establish whether temporary or persistet somatic variation can arise in clones obtained in vitro by micropropagation has been performed on *Cichorium intybus*. The results point out that persistent modifications of the phenotype can be observed after six cycles of cloning and that when the length of the cloning period is varied these appear to be differential responses of the same genotype to the micropropagation.
Key words. Cichorium intybus; clones, micropropagated; somatic variation.

A number of authors have clearly established that, in vegetatively propagated plants, differences in a given character may occur between the propagules of the clone. Such variations may arise during the first asexual propagation and then disappear after a period of time, as is observed with root characteristics of *Populus deltoides*[1] and in tiller number of *Lolium perenne*[2]. These temporary effects or 'c' effects, are generally ascribed to physiological or morphological influences arising from the specific environment in which the original plant developed[3]. On the other hand it has been observed that the above-mentioned variations may be persistent over further cycles of propagation[4,5] and Breese et al.[6], who found persistent variations in rates of tillering in *Lolium perenne*, inferred that intraclonal differences may be due to cytoplasmic or plasmon heterogeneities originated during the initial sub-cloning.

Though the nature of these extranuclear differences is not always easy to interpret, some authors[7–9] suggested that in asexually reproduced organisms differences could occur as result of degeneration of the cytoplasm during ageing.

The present investigation aims to establish whether temporary of persistent somatic variation can also arise in clones obtained in vitro by micropropagation, and whether the expression of several characters in one genotype can be altered by varying the lenght of the cloning period.

Materials and methods. Seeds of *Cichorium intybus* cultivar Chioggia were surface-sterilized by 30 min of treatment with 20% sodium hypochlorite.

They were then washed with sterile distilled water and placed on a medium with 3% sucrose solidified with 0.5% agar.

One week after germination the primary roots of six seedling were excised and discarded while the shoot tips were transferred to a medium stimulating the induction of adventitious shoots (modified Murasige and Skoog[10] which contained 0.3 mg/l GA, 0.01 mg/l NAA and 0.2 mg/l BAP) obtaining a population of ramets from each seed.

From these initial populations two types of clones were established differing from each other in the length of their propagation cycles, that is, one type was cloned every 14 days (short

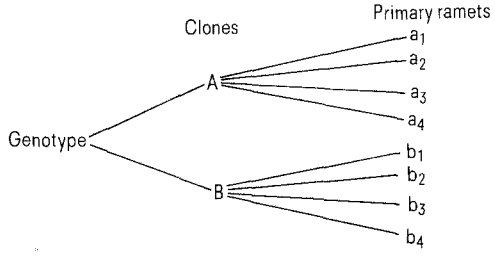
Table 1. Mean values and SE of the four traits analyzed on the A and B clones after the first and sixth cycles of cloning

Genotypes	Average number of leaf per plantlet				Plantlet height (mm)				Average number of leafs per plantlet				Rooting time (days)			
	A_1	A_6	B_1	B_6	A_1	A_6	B_1	B_6	A_1	A_6	B_1	B_6	A_1	A_6	B_1	B_6
1	5.2 ± 0.32	4.1 ± 0.22	4.7 ± 0.34	4.7 ± 0.27	28.9 ± 1.30	17.9 ± 0.76	29.4 ± 1.50	31.3 ± 1.20	18.2 ± 0.51	12.8 ± 0.32	18.6 ± 0.62	18.6 ± 0.48	7.6 ± 0.18	8.1 ± 0.22	6.4 ± 0.14	7.7 ± 0.18
2	4.5 ± 0.26	5.0 ± 0.23	4.2 ± 0.22	4.3 ± 0.29	29.7 ± 0.99	28.8 ± 0.75	26.2 ± 1.05	32.3 ± 1.02	19.4 ± 0.51	17.1 ± 0.31	15.7 ± 0.45	18.4 ± 0.44	7.2 ± 0.12	8.0 ± 0.23	6.9 ± 0.12	7.5 ± 0.10
3	3.19 ± 0.24	4.6 ± 0.24	3.7 ± 0.28	6.4 ± 1.16	38.3 ± 2.07	32.2 ± 1.28	22.9 ± 1.50	35.4 ± 1.22	25.8 ± 0.85	20.6 ± 0.54	15.5 ± 0.57	20.9 ± 0.45	8.6 ± 0.20	8.0 ± 0.15	8.15 ± 0.29	7.9 ± 0.14
4	4.3 ± 0.22	3.8 ± 0.24	4.0 ± 0.37	5.3 ± 0.50	35.1 ± 1.66	25.8 ± 1.08	23.2 ± 1.33	36.3 ± 1.61	22.7 ± 0.71	17.9 ± 0.48	15.4 ± 0.60	18.6 ± 0.49	7.6 ± 0.17	8.9 ± 0.25	7.4 ± 0.22	6.7 ± 0.14
5	4.2 ± 0.31	3.9 ± 0.18	4.3 ± 0.14	5.4 ± 0.30	34.9 ± 1.72	28.5 ± 1.20	30.3 ± 2.00	36.5 ± 2.00	19.6 ± 0.72	16.0 ± 0.47	15.5 ± 0.58	15.6 ± 0.51	7.5 ± 0.25	8.8 ± 0.31	7.1 ± 0.25	8.1 ± 0.19
6	4.8 ± 0.37	3.9 ± 0.21	4.0 ± 0.23	6.0 ± 0.37	38.3 ± 1.50	30.9 ± 1.15	27.1 ± 1.14	42.5 ± 1.37	25.4 ± 0.69	20.3 ± 0.52	17.3 ± 0.61	22.4 ± 0.52	7.3 ± 0.12	8.3 ± 0.22	6.8 ± 0.14	7.7 ± 0.17
Student's t-test for paired comparison	d.f. 5	t 0.39	d.f. 5	t 2.76*	d.f. 5	t 4.89**	d.f. 5	t 4.32**	d.f. 5	t 8.82**	d.f. 5	t 2.92**	d.f. 5	t 2.56*	d.f. 5	t 1.48

* $p < 0.05$; ** $p < 0.01$.

cycle = A clones) and the other every 28 days (long cycle = B clones); in order to reduce the 'c' effects[3], at each cycle the A and B propagules were taken at the 4–6 leaf stage, excised into segments each containing a node with an axillary bud, and allocated randomly in glass flasks containing the above described medium at 23 ± 1 °C constant temperature and under 4000 lux of fluorescent light with a 16-h photoperiod.

After the first cloning, both A and B clones from each genotype were divided into two groups: the former was maintained over six cycles, the latter was used to perform an experiment designed with the following nested scheme in order to evaluate the different components of the phenotypic variability:



Propagules belonging to the primary ramets were allowed to root on a Murasige and Skoog modified medium, containing 0.1 mg/l GA and 0.1 mg/l NAA. After 30 days, four traits were evaluated in the rooted plantlets; average number of leaves, leaf length, plantlet length and time of rooting expressed as number of days needed for the root to spring. In this way it was possible to estimate the variation associated with cloning (variance between primary ramets within clones) and the environmental variation (variance within primary ramets).

The above abridged experimental scheme was carried out again at the end of the sixth clonation.

Results and discussion. Mean values ($\bar{x} \pm SE$) of the four characters examined both at the first and the sixth propagation cycle are reported in table 1 for A and B clones. After the sixth cycle, while in A clones the mean values of plantlet height leaf length and rooting time show a significant decrease with respect to the same mean values observed at the first cycle, in B clones an increase of mean values of the four characters can be observed, which was not significant only for rooting time (table 1). In other words after six long cycles the B clones exhibit a larger phenotype and an accelerated rooting in comparison to the corresponding A clones after six short cycles.

Table 2. Hierarchical analysis of variance for A and B clones after the first (A_1, B_1) and sixth cycle (A_6, B_6) of cloning: s_a^2 = variance between primary ramets within clone; s_b^2 = variance within primary ramets; $d.f._a$ = degrees of freedom of s_a^2; $d.f._b$ = degrees of freedom of s_b^2.

	$d.f._a$	s_a^2	$d.f._b$	s_b^2	F
Average number of leaves per plantlet					
A_1	18	0.563	24	0.758	0.74
A_6	18	0.812	47	0.482	1.68
B_1	18	0.726	24	0.506	1.43
B_6	18	4.351	24	1.305	3.33**
Plantlet height					
A_1	18	565.064	452	181.092	3.12**
A_6	18	235.995	681	131.867	1.79**
B_1	18	406.838	448	152.598	2.66**
B_6	18	371.120	477	138.596	2.68**
Leaf length					
A_1	18	839.02	2079	127.420	6.58**
A_6	18	492.821	2976	99.705	4.94**
B_1	18	297.950	1935	91.700	3.25**
B_6	18	1077.228	2762	110.114	9.78**
Rooting time					
A_1	18	13.985	533	4.029	3.47**
A_6	18	8.813	538	4.563	1.93**
B_1	18	9.697	517	3.348	2.89**
B_6	18	8.036	517	2.195	3.66**

** $p < 0.01$.

In table 2 the results of the analysis of variance, computed at the first and the sixth cycle of clonation in A and B clones are reported. The estimates of variances from these hierarchical analyses show that the between primary ramets within a clone variance of the A and B clones is always significant both at the first and the sixth clonation. It is interesting to observe that

though the variability between primary ramets is maintained over six cycles of clonation, the trend of this variance is different in A and B clones. In fact we found that in A clones (short cycles) the variance between primary ramets for all the characters decreases from the first to the sixth clonation, while in B clones (long cycles) the various characters examined exhibit either an increase of variance or no modifications.

Summing up our results, the most impressive datum of this experiment is represented by the fact that, when the length of cloning period is varied, we observe a differential response of the same genotype to micropropagation, estimated on the characters considered as mean values and as between primary ramets as variability modifications.

As the significant variations observed between primary ramets at the first cycle are persistent after six cycles of vegetative reproduction, an explanation of our results on the basis of the 'c' effects[3] is untenable.

Considering that within A and B clones, belonging to the same initial population, the genotype should be constant, excluding spontaneous nuclear mutations which are too infrequent, the fact that these clones show different average phenotypes after six cycles of cloning might be explained on the basis of somatic variations due to cytoplasmatic inequalities which may have arisen during asexual reproduction[6]. However, if this were the case, we should observe a decrease of variability at the 6th cycle in both A and B clones, which conversely exhibit a different trend in the variability between primary ramets. Although we cannot exclude the presence of a certain amount of cytoplasmic heterogeneity, we may put forth that in cloning the same genotype at 14 and 28 days different developmental patterns could have been selected and consequently that the phenotypic variations observed in A and B clones could be due to modifications of gene expression.

In conclusion it is in our opinion that attention should be paid to the length of the cloning period of micropropagated plants in order to avoid undesirable phenotypes which seem to be related to environmental tresses, such as propagation periods which are too short.

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Mitotic cell cycle time in root meristems of tetraploid and related diploid pearl millet

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Summary. Mitotic cell cycle time was estimated from root meristems of tetraploid and related diploid stock (IP 1475) of pearl millet (*Pennisetum typhoides*). There is a 38% increase in cell cycle time in tetraploid meristems compared of their related diploid stocks.

Key words. Pearl millet; *Pennisetum typhoides*; mitotic cycle time; root meristem; tetraploidy.

The amount of cellular DNA, and DNA replication are known to have a direct effect on the mitotic cell cycle duration as measured in root meristems of different plant tissues[2]. Increased DNA content per cell may affect cell cycle duration[3]. A comparison of polyploids and related diploid species yielded varying results: the cell cycle duration was sometimes shortened[4], sometimes prolonged[5] and sometimes unaffected[6]. In rye[7] and barley[8] the mitotic cell cycle is prolonged in autotetraploids when compared to that in diploids.

In pearl millet, related populations are available which differ in total nuclear DNA content. The results of a comparison of diploids with and without B chromosomes have been previously reported. The difference between non B and 3B plants was small but the plants with 5B chromosomes showed a significant increase of 39% in cell cycle time compared to non-B stocks[9]. The mitotic cell cycle time in embryo and endosperm tissue of diploids and tetraploids was estimated by Krishna Rao and Aswani Kumari[10]. The present work deals with the cell cycle time in root meristems of diploid and autotetraploid stocks of pearl millet.

Materials and methods. Seeds of a diploid inbred line IP 1475 and its induced autotetraploid in the C_{10} generation were soaked for 2 h in tap water and placed on moist filter papers in petridishes at 30±2°C for germination. After 32 h seedlings with roots were immersed in 0.1% colchicine for 1 h and then thoroughly washed in running water. Later they were placed on a moist filter paper. Root tips were fixed for estimating mitotic cell cycle time starting from 1 h after colchicine treatment from both diploid and tetraploid seedlings. Root tips were sampled at 1 and 2 h and regularly at 2 h intervals thereafter up to 32 h after colchicine treatment. Roots were fixed in 1:3 acetic acid-methanol mixture and transferred to 70% alco-