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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1 Wilcox, J.R., and Farmer, R.E., Heritability and 'C' effects in early root growth of eastern cotton wood cutting. Heredity *23* (1968) 239.
2 Hayward, M.D., and Koerper, F.D.W., Environmentally induced variability in *Lolium perenne*. Z. PflZücht. *70* (1973) 195.
3 Libby, W.J., and Jund, E., Variance associated with cloning. Heredity *17* (1962) 533.
4 Darlington, C.D., Evolution of Genetic Systems, 2nd edn. Oliver and Boyd, London 1958.
5 Palenzona, D.L., Cavicchi, S., and Micardi, L., Irreversible phenotypic changes in *Phleum* associated with cloning in different environments. Proc. International Meeting on Quantitative Inheritance, Polymorphisms, Selection and Environment. Bologna, Italy 1973.
6 Breese, E.L., Hayward, M.D., and Thomas, A.C., Somatic selection in perennial ryegrass. Heredity *20* (1965) 367.
7 Beddows, A.R., Breese, E.L., and Lewis, B., The genetic assessment of heterozygous breeding material by means of a diallel cross. Heredity *17* (1962) 501.
8 Jinks, J.L., Extrachromosomal inheritance. Prentice-Hall, Inc., N.J. 1964.
9 Mather, K., Age of parent and character of offspring. Nature *174* (1954) 304.
10 Murasige, T., and Skoog, F., A revised medium for rapid growth and bio-assays with tobacco tissue cultures. Physiol. Plant. *15* (1962) 473.

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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-

hol after 24 h. The 2×, 4×, 8× metaphases were scored from diploid and tetraploid treated root tips. For each interval three slides were prepared from three root tips and from each slide approximately 800–1000 cells were scored from Feulgen preparations.

Results and discussion. Mitotic cell cycle time was estimated as the interval between two successive divisions of a selected synchronous cell population, i.e. C-metaphases that emerge as tetraploid cell population at the time of the next division, seen as 4× cells in 2× roots and 8× cells in 4× roots. The percentage cells with C-metaphases was scored 1 h after treatment and subsequently at 2 h intervals until 32 h.

The proportion of C-metaphases in diploid pearl millet 1 h after treatment was 7.7%. A comparable percentage of 4× metaphases was observed only in the sample taken 22 h after the treatment. Thus the mitotic cell cycle time of diploid (IP 1475) pearl millet was estimated to be 21 h (22–1 h).

The proportion of C-metaphases in tetraploid pearl millet 1 h after treatment was found to be 7.8%. The highest proportion of C-metaphases at the 8× level was only 6.8%. This level of accumulation of 8× metaphases was found only in the sample taken 30 h after the treatment; therefore, the cell cycle time of tetraploids was estimated to be 29 h. Thus, the difference in mitotic cell cycle time of the diploid and its autotetraploid amounts to 8 h, i.e. an increase of 38%.

Comparison of mitotic cell cycle times in embryonic tissue of pearl millet diploids and tetraploids also revealed a small difference; the duration was longer in 2n embryos[10]. However, the same study using vg 212, IP 482, IP 482T and C_8 HF lines revealed no difference in mitotic cell cycle time in endosperm of diploid and tetraploid lines. They suggested that the effect of polyploidy can be different on different cell types within the same species. Results of the present experiment with root meristems, while agreeing with this general conclusion, further demonstrate that 1) the tetraploid root meristems show prolonged mitotic cell cycle compared to their related diploid and 2) that the cycle time in a more mature tissue (i.e. radicle) is longer than that in the embryonic tissue (early embryo)[11].

Frequencies of metaphases expressed as percentages at first and second divisions of root meristems of diploid (IP 1475) and tetraploid pearl millet

Sampling time (h)	Metaphases 2n		4n	
	2×	4×	4×	8×
1	7.70		7.80	
2	8.25		7.13	
4	8.50		1.40	
12	4.85		1.00	
14	1.90		0.50	
16	1.10	0.30	0.90	
18		0.90	0.60	
20		6.00	0.20	
22		7.38		
24		2.25		1.10
26		1.40		1.70
28		1.10		3.10
30				6.80
32				1.10

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2 Van't Hof, J., Exp. Cell Res. *39* (1965) 48.
3 Pallitti, F., Rocchi, A., Mercanti, A., and Olivieri, G., Caryologia *25* (1972) 365.
4 Evans, G.M., Rees, H., Snell, C.L., and Sun, S., Chromosomes today, vol. 3, p. 24. Eds C.D. Darlington and K.R. Lewis. Oliver and Boyd, Edinburgh 1972.
5 Yang, D., and Dodson, E.O., Chromosoma, Berl. *31* (1970) 309.
6 Gupta, S.B., Can. J. Genet. Cytol. *11* (1969) 133.
7 Fedeyeva, T.S., and Shakhla, Z.H., Tsitologia *16* (1974) 698.
8 Skult, H., Acta Acad. aboensis *B29* (1969) 1.
9 Krishna, Rao, M., Pantulu, J.V., and Jayalakshmi, K., Experientia *35* (1979) 1154.
10 Krishna Rao, M., and Aswani Kumari, K., J. Cytol. Genet. *15* (1980) 134.
11 Avers, C.J., Cell Biology, p. 452. D. Van Nostrand Co., New York 1976.

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Lack of mutagenicity of irradiated glucose in *Salmonella typhimurium* using host-mediated assay

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Summary. Experiments were conducted to study the ability of irradiated glucose to induce reverse mutations in *S. typhimurium* by host-mediated assay. The results revealed no significant increase in the frequency of reverse mutations compared to controls.
Key words. Salmonella typhimurium; glucose, irradiated; mutations, reverse; host-mediated assay.

Preservation of food and food components by radiation offers several advantages in reducing spoilage. However, the suitability of the resulting products for human consumption is debatable in view of conflicting results on genetic hazards of irradiated food stuffs reported by several investigators[1–4]. Carbohydrates being one of the main components of food material, greater importance was attached to the studies on the effects of irradiation on sucrose and glucose in particular. Mutagenic effects of irradiated sugar solutions were first indicated by Ehrenberg[4] on barley. Subsequently, other investigators reported positive evidence of mutagenic effects in plant and mammalian cells after treatment with irradiated sugar solutions[6,7]. Host-mediated assay is a valuable test system which determines the ability of the host to detoxify the test compounds with regard to their mutagenic activity[8]. Using this test system, experiments were undertaken to assess the mutagenicity of irradiated glucose using mice as hosts and *Salmonella typhimurium* G-46 as indicator organism.