PRO EXPERIMENTIS

A simple method for determination of the proportion of 2C and 4C cells in a dormant embryo

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Summary. Pea embryos grown in the presence of 2.5 mM hydroxyurea from the beginning of imbibition showed a mitotic peak the height of which was considerably less than that of the control material. Soon after the mitotic peak, there was a complete cessation of mitosis in the treated material. The extent of suppression of mitosis during the first post-dormancy cell cycle caused by hydroxyurea has been used to obtain an estimate of the relative proportion of 2C and 4C cells in the embryo.

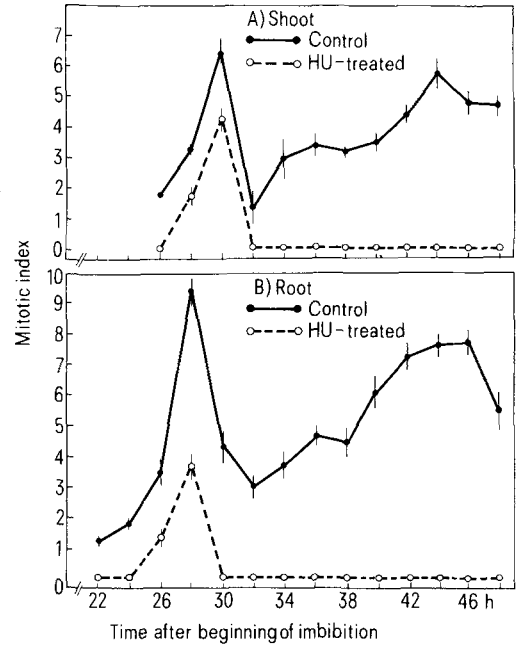
Recently we have discussed the occurrence of 2C and 4C cells in dormant angiosperm embryos and emphasized the need for examining the 2C-4C cellular composition of the dormant embryo together with a study of its early biochemical activities[2]. 3 methods have been generally used for the determination of the relative proportion of 2C and 4C cells in a dormant embryo. These are: Feulgen microspectrophotometry, H^3-thymidine autoradiography, and the response of dormant chromosomes to ionizing radiation. We describe here a simple and inexpensive additional method. It is based on the use of the chemical hydroxyurea (HU). Our test material is the post-dormancy cycling cell population of pea root tip and the terminal portion of the emerging shoot. The dormant pea root tip has been shown previously by H^3-thymidine autoradiography to consist of a mixture of 2C and 4C cells[3].

Hydroxyurea is known to inhibit DNA synthesis, kill S-phase cells and prevent G_1 (2C) cells from entering S-phase both in vitro[4-8] and in vivo[9-12]. It appears to have little or no effect on the survival and progression of cells in other phases of the cell cycle[4,5,13,14]. If the chemical exerts similar effects in plants and does not act differentially on cells in the same phase of the cell cycle one would expect that in a 2C embryo which has been grown in the presence of HU there should be no mitosis during early germination. However, in a 2C-4C embryo grown similarly there will be some initial mitoses arising from the progression of 4C cells to be followed by a complete cessation of mitosis coinciding with the depletion of the 4C cells. In such an embryo the comparison of the mitotic indices (percentage of cells in mitosis) of control and HU-treated material should provide an estimate of the relative proportion of 2C and 4C cells.

Pea seeds were placed in vermiculite in wide-mouthed screw cap glass jars and moistened with distilled water (control) or a solution of 2.5 mM HU and the seeds were allowed to germinate at 25 ± 1 °C. The quantity of vermiculite, the number of seeds and the amount of water or HU solution used to moisten the vermiculite were strictly controlled in each jar. It had been determined previously that a concentration of 2.5 mM HU could effectively suppress mitotic activity in an actively proliferating meristem by nearly 80% within 6 h. At different times after the beginning of imbibition root tips and terminal portions of the emerging shoot were selected for uniformity by visual examination and fixed in ethanol-acetic acid, 3:1. They were Feulgen stained after 14 min hydrolysis in 1 N HCl at 60 °C in a circulating water bath. For each fixation time at least 4 root tip squashes and 4 shoot squashes were made separately and from each squash preparation at least 500 cells were scored to determine the mitotic index.

The figure shows the pattern of mitotic activity in the control and HU-treated material. In both root tip and shoot there occurs a pronounced mitotic peak which indicates the existence of a partially synchronous cell population. This is in accord with our other results on the same material. The root tips of some early fixations gave relatively uniform mitotic index values compared with those of later fixations. The uniformity of the sample is probably due to the fact that in these early stages differentiation has not yet started.

In the figure, the difference in the height of the mitotic peak between the treated and the control material could result from 1 of 2 causes. First, on the assumption that the peak is caused solely by 4C cells then the reduced height in the treated material would suggest that some of these 4C cells are sensitive to HU while some are not. Alternatively, the peak height of the control material may be the contribution of both 2C and 4C cells and the reduction of the height in the treated material may indicate that HU blocked the 2C cells at the G_1 - S boundary thereby causing a reduction in the peak height. This interpretation would loose its validity if the situation is such that the 2C cells enter the first mitosis long after the 4C cells have done so. But in this material both 2C and 4C cells arrive at the early mitosis simultaneously as indicated by the occurrence of both labelled and unlabelled mitoses during early hours of germination in the data of Bogdanov and Jordansky[3]. Thus, the above situation does not exist in this material. If the former interpretation is accepted then the results would indicate that in the dormant embryo there is a substantial number of 4C cells, some sensitive to HU and some



Effect of hydroxyurea on the pattern of mitotic activity in the emerging shoot (A) and the root meristem (B) of pea during early hours of germination.

insensitive. On the other hand, the reported insensitivity of 4C cells to HU as discussed above would add credence to the latter interpretation and consequently the reduced peak height would be indicative of the relative proportion of 2C and 4C cells. Thus, the ratio of the peak height between the treated and the control material (approximately 0.4 for the root tip) would imply that in the root tip for example about 40% cells were blocked by HU and these are presumably 2C and the remaining 60% are 4C. By this criterion, the shoot appears to contain 60% 4C and 40% 2C cells in the dormant cell population. These estimates are in good agreement with a previous report[3] which indicates that approximately 50% of the dormant cycling cells in the pea root tip are in the 4C state. As expected, HU completely abolished mitosis soon after the 1st peak and this was maintained as long as the sampling continued.

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CORRIGENDUM

F. Yasuda and H. Tada: Desacetylscalaradial, a cytotoxic metabolite from the sponge *Cacospongia scalaris*, Experientia *37*, 110 (1981). Page 111, line 8 right column, ... between 18-H and 20-H suggested ... should read ... between 18-H and **16-H** suggested that ...

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