Abstract. Developing lateral roots of *V. faba* were treated with 5-aminouracil for up to 6 hours using the 5-AU inhibition method discussed in this paper; the duration of \( G_2 + \frac{\text{mitosis}}{2} \) and the percentages of slow dividing cells were estimated from the fall in MI observed in just emerged meristems, very large primordia and large primordia. The results indicate that during the period of development studied here there are two subpopulations of dividing cells: 1) fast dividing population which makes up about 84% of the dividing cells and which has a \( G_2 + \frac{\text{mitosis}}{2} \) duration of about 3.3 hours, and 2) a slow dividing population which constitutes about 16% of the dividing cells and which has a \( G_2 \) duration in excess of 12 hours. This heterogeneity is discussed in relationship to the behaviour of different populations of proliferating cells during root morphogenesis.

Introduction

Mitotic cycle duration and the duration of its phases (\( G_1 \), \( S \), \( G_2 \) and mitosis) change in proliferating populations during development. In animals the rate of increase in cell number shows that cell cycle duration increases as an embryo ages. Using the percentage labeled mitosis method (Quastler and Sherman, 1959) it has been shown that a similar change occurs, in plants, during lateral root morphogenesis. The mean cycle duration of the fast dividing population of cells is 2 to 2.5 hours shorter in primordia with \(<1,500\) cells than in cells in the fast dividing population of lateral roots of *Vicia faba* (MacLeod and Davidson, 1968; Webster and Davidson, 1968). Since in some stages of lateral root development in *V. faba* the cells either do not incorporate exogenously supplied \( \text{H}^3\text{-TdR} \) (MacLeod and Davidson, 1968) or they show sporadic labeling or low levels of labeling (Friedberg, personal communication; Socher, unpublished), the percentage labeled mitosis method cannot be used to measure cycle parameters in these stages of lateral root development. However, one or more phases of the cycle can be estimated by other methods.
Treatment with 5-aminouracil (5-AU) can be used to measure the duration of $G_2 + \frac{\text{mitosis}}{2}$ in proliferating systems (Socher and Davidson, 1970). In the experiments to be reported here the drop in mitotic index during 5-AU treatments has been used to estimate (a) the duration of $G_2 + \frac{\text{mitosis}}{2}$ and (b) the degree of population heterogeneity in several stages of lateral root development in *V. faba* that show atypical labeling patterns following a pulse exposure to $^{3}H$-TdR. The results indicate that from the time a primordium consists of 1,500 cells until it is fully emerged as a lateral root two populations of dividing cells are present and they differ in the duration of their $G_2$ period of interphase.

**Materials and Methods**

Beans of *Vicia faba* L. were germinated and grown as previously described (Socher and Davidson, 1970). Roots were treated for up to 6 hours with 500 ppm (3.93 × $10^{-3}$M) and 1500 ppm (1.18 × $10^{-2}$ M) 5-AU (Nutritional Biochemicals Corp., Cleveland, Ohio). Treated and control roots were fixed every hour from 2 to 6 hours.

Roots were fixed in acetic acid-absolute alcohol (1:3) containing a few drops of formalin. Roots were washed, hydrolyzed in 1 N HCl at 60° for 9 minutes and stained with Feulgen. Primordia were dissected out and prepared as squashes.

The following developmental stages were examined:

a) Just emerged meristems: These are roots of which only the meristematic region has broken out through the epidermis.

b) Very large primordia: These are primordia that are about to emerge but have not broken through the outer 2 or 3 layers of cortex and the epidermis. They are cone- or dome-shaped.

c) Large primordia: These are primordia of more than 1,500 cells. They are smaller than very large primordia and have a rounded shape.

**Results**

When roots are treated with 5-AU for up to 6 hours there is a progressive decrease in mitotic index (MI) with time. The pattern of the drop in MI shows a striking similarity in lateral roots (Socher and Davidson, 1970), just emerged meristems, very large primordia and large primordia (Fig. 1). There is also a regular progression in the disappearance of cells from the various stages of mitosis (Table 1). For example, in large primordia, the drop in MI observed at 2 and 3 hours of 5-AU treatment is due almost entirely to a reduction in the frequency of cells in prophase. A significant reduction in the frequency of cells in metaphase and in anaphase is not detected until after 4 hours of treatment and a drop in the frequency of telophase is observed only at 5 hours. In addition, the number of cells in prophase and metaphase remains con-